Title Page

Clinical Study Protocol

A Single-Center, Open-Label, Dose-Escalation Phase I Clinical Trial of Recombinant Human Granulocyte Colony Stimulating Factor-Fc Fusion Protein for Injection as an Adjuvant to Chemotherapy in Subjects with Breast Cancer

Protocol Number:	**2012-F-627-CH1**	
Investigational Drug:	Recombinant human granulocyte colony stimulating factor-Fc fusion protein (F-627)	
Clinical Phase:	Phase I	
Current Version No.:	Amendment 1.0	
Issue Date of Current Version:	May 13, 2013	
Original Version No./ Issue Date:	Final Version: 2.5/Dec. 3, 2012	
Study Site:	Fudan University Shanghai Cancer Center Address: No. 270 Dong'an Road, Shanghai, China	Postal Code: 200032
Principal Investigator	Professor Cao Junning	Tel.: +86-021-61733900
Sponsor	Generon (Shanghai) Corporation Ltd. Address: Building 9, No. 787 Kangqiao Road, Shanghai, China	Postal Code: 201315
Contact	Huang Cheng	Tel.: +86-021-61760866

Summary of Amendments of Protocol

Amendments of this protocol include:

1. In Section 4.1, "dose delay and one-time reduction due to toxicities other than myelotoxicity (such as cardiotoxicity) are permitted" was changed to "Dose delay and one dose reduction due to chemotherapy-induced toxicities (such as cardiotoxicity) are permitted".

2. Item 4 of the inclusion criteria "Absolute neutrophil count (ANC) $\geq 2.0 \times 10^9$/L, hemoglobin (Hb) ≥ 11.5 g/dL, and platelet (PLT) $\geq 100 \times 10^9$/L prior to chemotherapy" was changed to "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 chemotherapy".

3. Exclusion criteria 11 "history of TB; or history of TB exposure, unless negative for tuberculin test; TB patients undergoing treatment; or suspected TB evaluated by chest x-ray" was changed to "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".

4. The window for examination on the first day of cycles 2–4, namely day 22, day 43 and day 64 of the study, was set to 3 days.

5. Administration time of the study drug was changed from morning (08:00 ± 1:00) to morning (09:00 ± 1:00).

6. Blood sampling points were added on day 16 (± 24 hrs) and day 21 (± 24 hrs) for pharmacokinetics (PK) in cycle 1 and cycle 3.

7. Time points for serum antibody collection were changed from each day 8, day 13 and day 21 in cycles 1–4 to each day 1, day 8 and day 13 in cycles 1–4 and day 22 in cycle 4.

8. "The recommended concentration of F-627 is 2.0 mg/mL" was deleted from Section 5.5; "See the drug preparation procedure manual" was added.

9. In Section 6.2, "For chemotherapy cycles 2–4 (day 3–day 21 of each chemotherapy cycle, i.e., day 23–day 84 of the study), starting on each day 2 of cycles 2–4, oral temperature measurement and routine blood test will be performed every other day" was changed to "For chemotherapy cycles 2–4 (days 3–21 of each chemotherapy cycle, i.e., days 23–84 of the study), starting from day 3, oral temperature measurement and routine blood test will be performed every other day".

Signature Page

A Single-Center, Open-Label, Dose-Escalation Phase I Clinical Trial of Recombinant Human Granulocyte Colony Stimulating Factor-Fc Fusion Protein for Injection as an Adjuvant to Chemotherapy in Subjects with Breast Cancer

Protocol Number: 2012-F-627-CH1

I confirm that I have read, understood and agreed to abide by all provisions in the Clinical Study Protocol (Number: 2012-F-627-CH1, Date: May 13, 2013). I shall carefully carry out my duties in accordance with the Good Clinical Practice.

Principal Investigator

曹军宁 2013.5.28

Cao Junning
Signature Date

曹军宁

Cao Junning
Principal Investigator (Printed Name)

Sponsor

黄2186 2013-5-20

Huang Cheng
Signature Date

黄2186

Huang Cheng

Emergency Contacts of Generon (Shanghai) Corporation Ltd.

In case of a medical emergency, the clinical study leading official may be contacted. If the director is not available, the protocol writer or the clinical research associate (CRA) may be contacted via the information shown in the table below.

	Name	Contact Number
Project leading official	Huang Cheng	021-6176-0866 or 18621697558
Protocol Writer	Tang Kaiyang/Huang Cheng	021-6176-0866 or 18621697558
24-Hour Emergency Contact Number	Huang Cheng	021-6176-0866 or 18621697558

Synopsis of Phase I Clinical Study Protocol

Investigational Drug	Recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection
Protocol No.	**2012-F-627-CH1**
Title	A single-center, open-label, dose-escalation phase I clinical trial of recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection as an adjuvant to chemotherapy in subjects with breast cancer
Mechanism of Drug Action	The recombinant human granulocyte colony stimulating factor (rhG-CSF)-Fc fusion protein is a long-acting rhG-CSF expressed in CHO cells. Each molecule of rhG-CSF-Fc fusion protein contains two G-CSF dimer molecules, which may overcome the weak bioactivity of pegfilgrastim and produce a stronger receptor activation signal, thereby accelerating the differentiation and proliferation of neutrophils in bone marrow. Also, preclinical studies have shown that the pharmacokinetic and pharmacodynamic properties of rhG-CSF-Fc is different from or superior to pegfilgrastim, and therefore it decreases the severity of neutropenia and reduces the duration of severe neutropenia in cancer patients after chemotherapy.
Study Site	Fudan University Shanghai Cancer Center
Pharmacokinetic Analysis Site	Covance Pharmaceutical Research and Development (Shanghai) Co., Ltd.
Number of Planned Enrollment	It is planned to enroll 18 subjects
Study Population	Female postoperative patients with breast cancer who receive adjuvant chemotherapy will be enrolled in this study. Eligible patients should have received no chemotherapy or only one chemotherapy. The chemotherapy used for this trial is EC→P or EC→T, 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 all 4 cycles, subjects will receive 4 subsequent cycles of sequential chemotherapy (either paclitaxel or docetaxel) and supportive care according to routine practice.
Objectives	**Primary Objective:** To evaluate the safety and tolerability of recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection (F-627) in treatment of female postoperative breast cancer patients who require adjuvant chemotherapy in a single-center, open-label, single-dose and repeated-dose, dose-escalation phase I clinical trial.
	Secondary Objectives: 1. To evaluate the pharmacokinetics of F-627 by determining the serum drug concentrations of F-627 at different time points after dosing; 2. To evaluate the pharmacodynamics of F-627 by analyzing the relationship between serum drug concentrations of F-627 and neutrophil counts in blood at different time points after dosing, and to provide a recommended dose for phase II clinical trial.
	Exploratory Objectives: 1. To observe the number of days in which absolute neutrophil count is less than 0.5×10^9/L in cycle 1 of chemotherapy, the average time of ANC recovery, and the number of days in which absolute neutrophil count is less than 1×10^9/L, and to provide guiding significance for phase II clinical trial. 2. To evaluate the potential immunogenicity of F-627 by testing anti-F-627 antibodies (IgG and IgM) in serum.

Dosage and Administration	This study includes 3 dose cohorts, 80, 240, and 320 μg/kg, each of which will enroll 6 patients with breast cancer sequentially receiving adjuvant chemotherapy. Subjects in each cohort will receive a single-dose of F-627 by subcutaneous injection approximately 48 hours after the completion of chemotherapy. Blood samples are then collected at multiple time points during the subsequent follow-up visits, to evaluate the pharmacokinetics, pharmacodynamics, and safety of the drug. If dose-limiting toxicities are not observed before the start of cycle 2, then the same dose of F-627 is to be given at approximately 48 hours after each chemotherapy in cycles 2-4.
Dose-Escalation	The starting dose is 80 μg/kg. Only after 6 subjects have completed the treatment and observation of the first cycle, the sponsor and investigator will determine whether to proceed to the next higher dose based on the safety evaluation. This is also applicable to the 240 μg/kg cohort. Dose escalation is not pursued after 6 subjects in the 320 μg/kg cohort complete the evaluation. Dose escalation should be stopped if 2 cases of DLTs are observed in the first cycle in each cohort.
Rationale for Dose Selection	Results from 3-month repeat dosing toxicity studies showed that the no-observed-adverse-effect-levels (NOAEL) of F-627 was 1000 μg/kg in rats and 675 μg/kg in cynomolgus monkeys. F-627 is a recombinant fusion protein with a molecular weight of 95000 Daltons. Results from preclinical studies showed that the toxicity of F-627 was due to the amplification of pharmacodynamics, which is closely associated with the dose (expressed in mg/kg) in various different species. The maximum safe starting dose of F-627 was calculated to be 67.5 μg/kg when converted using mg/kg, which is one-tenth of the NOAEL in cynomolgus monkeys. In a phase I clinical trial in healthy volunteers, male subjects received a single-dose of F-627 by subcutaneous injection at doses of 30, 60, 120, 240, and 360 μg/kg. Results showed that neutrophil increased across all dose cohort up to 96 hours following the administration of F-627. The maximum dose of 360 μg/kg was safe and no serious adverse events observed. Higher incidence rate of adverse event was observed with the increasing dose. Low dose was better tolerated than high dose. According to the animal studies and phase I clinical trial, 80 μg/kg is safe as the starting dose for patients. Firstly, the maximum dose should not exceed the maximum dose determined in the phase I clinical trial. Secondly, the G-CSF mole number of 240 μg/kg F-627 is comparable to that of 100 μg/kg pegfilgrastim. Finally, 60 μg/kg cohort exhibited superior pharmacodynamics than the 150 μg/kg cohort in treating the chemotherapy-induced neutropenia in monkey models.
Dose-Limiting Toxicity	Dose-limiting toxicity (DLT) refers to an intolerable toxicity that is experienced during the treatment with investigational drug and limits the further dose escalations. DLT is defined as any grade 3 or greater adverse event related to the investigational drug that observed in cycle 1 (21 days). Adverse events will be assessed according to NCI CTCAE V4.03 criteria. After 6 subjects in each dose of F-627 cohort have completed the treatment and observation of first cycle, the trial may proceed into the next dose cohort only if less than 2 subjects develop DLT. If a grade 3 or greater adverse event related to the investigational drug is observed in cycles 2-4, then the investigator and sponsor will determine together whether the adverse event would affect further dose escalations.
Safety Evaluation	Safety endpoints include laboratory measurements, physical examinations, vital signs, and performance status, as well as adverse events. The incidence rate and severity of adverse events (AEs) will be assessed according to NCI CTCAE 4.03 criteria.
Salvage Therapy	During the study, an approved G-CSF treatment should be given to the patient as salvage therapy in the case of febrile neutropenia (defined as ANC $< 1.0 \times 10^9$/L; a single measurement of body temperature > 38.3 °C or a fever ≥ 38.0 °C sustained for longer than 1 h; Note: temperature measurements is based on oral temperature or equivalent armpit/rectal temperature) or grade 4 neutropenia lasting greater than 3 days. The sponsor recommends GRAN® (filgrastim) at a dose equivalent to 5 μg/kg/day of G-CSF, once daily for ≤ 2 weeks or until neutrophil count recovers to 1.0×10^9/L.

Inclusion Criteria	All of the following conditions must be met: 1) 18-75 years old; 2) Female postoperative breast cancer patients who require adjuvant chemotherapy, and are planned to receive 4 cycles of EC chemotherapy; 3) ECOG performance status of 0-1; 4) 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 chemotherapy 5) Hepatic and renal function within the normal range; 6) Left ventricular ejection fraction greater than 50%; 7) Willing to sign the informed consent form and able to comply with protocol requirements;
Exclusion Criteria	Subjects who meet any of the following must be excluded from this study: 1) Women in pregnancy or breastfeeding; Women of child–bearing potential have a positive pregnancy test result prior to the first dose; 2) Life expectancy less than 12 months; 3) Radiation therapy within 4 weeks prior to enrollment; 4) Breast cancer patients who have received neoadjuvant chemotherapy before radical mastectomy; 5) Prior bone marrow or stem cell transplant; 6) With other malignant tumors other than breast cancer; 7) Have received G-CSF treatment within 6 weeks prior to enrollment; 8) Diagnosed with acute congestive heart failure, cardiomyopathy, or myocardial infarction by clinical diagnosis, ECG or other approaches; 9) With any disease that may cause splenomegaly; 10) With acute infection, chronic active Hepatitis B within 1 year (unless patients tested negative for HBsAg prior to enrollment), or Hepatitis C; 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) Known HIV positive or AIDS; 13) With sickle cell anemia; 14) With alcohol or drug abuse that may affect the compliance with the study; 15) With known hypersensitivity to E. coli derived proteins, G-CSF, or excipients; 16) Has received any other investigational drug within 4 weeks prior to enrollment; 17) Patients with diseases or symptoms unsuitable for participating in the clinical trial based on the investigator's judgment;
Criteria for Terminating Study	1) Incidence rate and severity of serious adverse events (SAEs) indicate the study should be terminated early as determined by the investigators and the sponsor; 2) The dose-limiting toxicity of the subjects does not recover or cannot be relieved; 3) The investigators question the safety of the drug and believe that the continuation of the study may pose serious risks to the subjects; 4) The maximum dose set in the clinical trial has been reached; 5) Data fraud, or inaccurate/incomplete collection of data;
Criteria for Subject Withdrawal	Subjects will withdraw from the study with a written explanation when the following conditions occur. 1) Subject withdraws voluntarily during the trial; 2) The investigator considers that withdrawal is for the best interest of the subject; 3) The investigator or subject believes that to continue the trial may result in intolerable adverse events; 4) Complications or worsening co-morbidities affecting subject's participation occur; 5) Subject is found to violate protocol after enrollment, or a major protocol violation occurs during the trial; 6) When a safety concern regarding the investigational drug arises, but data is yet unknown, resulting in potential risks if subjects continue the trial;

Drop-Out Criteria	Determination of drop-outs: Eligible subjects who have signed the informed consent form and been enrolled have the right to withdraw from the study at any time. Subjects who do not complete the entire observation are considered drop-outs regardless of the time or reason of the withdrawal. A replacement should be implemented immediately according to the original plan when a drop-out occurs. Blood samples from drop-out subjects should be retained.
Evaluations:	
Safety Evaluation	Medical consultation, physical examinations, vital signs, laboratory measurements (hematology, clinical chemistry, routine urinalysis, etc.), weight, ECG, abdominal ultrasound and adverse event evaluation.
Pharmacokinetic Evaluation	Serum: C_{max}, T_{max}, MRT, V_d, Kel, Tl/2z, AUC_{last}, AUC, CL/F, Vz/F
Pharmacodynamic Evaluation	Absolute neutrophil count (ANC) after dosing, as well as the number of days in which ANC is less than 0.5×10^9/L, the number of days in which ANC is less than 1.0×10^9/L, and the time ANC recovers to 1.0×10^9/L after chemotherapy in cycles 1 and 2-4 are observed.
Statistical Analysis: Descriptive statistics will be summarized for all variables obtained at various observation time points by dose cohorts, unless the protocol specifies that statistical analysis at a particular time point is not required. Overall, continuous variables (such as age) will be descriptively summarized with observed numbers, mean, median, standard deviation, minimum, and maximum. Categorical variables will be descriptively summarized with frequency and percentage based on each category. Continuous safety analysis of adverse events related to the investigational drug and other safety endpoints will be performed. The analysis dataset includes all enrolled subjects who have received at least one dose of the study drug. This dataset is available for all analyses. Statistical methods are detailed in the Statistical Analysis Plan.	
Pharmacokinetics	Two phases (single-dose and repeated dose); All PK parameters will be summarized using descriptive statistics by dose cohorts. The non-compartmental analysis of blood concentration data will be performed by the central laboratory using WinNonlin Enterprise. C_{max} (natural logarithm), $AUC_{(0\text{-inf})}$, AUC_t, and AUC_{last} of the single-dose and repeated-dose phase were analyzed using one-way ANOVA, with dose cohort as the fixed factor. The least squares mean difference between the two dose cohorts and the 90% confidence intervals are obtained from the analysis of variance, then the least squares geometric means ratio and the 90% confidence intervals are obtained by taking the antilog.
Pharmacodynamic Evaluation	The number of days in which ANC is less than 0.5×10^9/L, the number of days in which ANC is less than 1×10^9/L, and the mean time of ANC recovery to 1.0×10^9/L in chemotherapy of cycles 1 and 2-4 of chemotherapy are calculated for each dose cohort. ANC profiles in various dose cohorts are plotted.
Safety Evaluation	All adverse events are listed by patient, and coded by MedDRA as per System Organ Class and Preferred Term. Inferential statistics are not required for safety endpoints. Abnormal values of laboratory measurements, vital signs, ECG, and other safety endpoints need to be noted. The incidence rate and severity of adverse events (AEs) will be assessed according to NCI CTCAE 4.03 criteria.

Table of Contents

List of Tables

List of Figures

Abbreviations and Terms

Abbreviations and Terms	Definitions
AEs	Adverse Events
ALP	Alkaline Phosphatase
ANC	Absolute Neutrophil Count
AUC	Area Under Concentration-Time Curve
CL/F	Total Clearance
C_{max}	Maximum Serum Concentration
DHL	Lactate Dehydrogenase
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ED_{50}	Median Effective Dose
EMEA	European Medicines Agency
FDA	U.S. Food & Drug Administration
GCP	Good Clinical Practice
Kel	Elimination rate constant
MRT	Mean Residence Time
NCI CTC	National Cancer Institute Common Terminology Criteria
NOAEL	No-Observed-Adverse-Effect Level
PK/PD	Pharmacokinetics/Pharmacodynamics
Pubmed	PubMed search system developed by the National Institutes of Health
rhG-CSF-Fc	Recombinant human granulocyte colony stimulating factor-Fc fusion protein
SFDA	State Food and Drug Administration
T_{max}	Time to Peak
V_d	Apparent Volume of Distribution
V_z/F	Volume of Distribution
WBC	White Blood Cell
WHO	World Health Organization

1. STUDY BACKGROUND

1.1 Introduction to F-627

1.1.1 Name, structure, and physicochemical properties

All recombinant human granulocyte colony stimulating factors (rhG-CSF) that have already been approved for marketing in China and other countries, and the more than 10 rhG-CSFs in clinical and pre-clinical studies, are monomeric G-CSFs (Pharmaproject ref). This rhG-CSF-Fc fusion protein, is a rhG-CSF dimer expressed in CHO cells, based on the technology of Fc fusion, whose IP is protected by Chinese patents. The Fc fragment is derived from human immunoglobulin with prolonged effects. The half-life of IgG immunoglobulins may be up to 3 weeks in human blood. Recombinant protein drugs generated using the Fc fusion protein technology such as Enbrel (for rheumatoid arthritis, soluble TNFα receptor and IgG_1) and chimeric monoclonal antibodies such as Remicade (for rheumatoid arthritis, anti-TNFα IgG_1) and Abciximab (for platelet aggregation, anti-GPIIb/IIIa IgG_1) have been successfully marketed. In addition, D-Mab (for osteoporosis, anti-RANKL IgG_2) has been approved for marketing by FDA. The safety and efficacy of Fc fusion protein have been validated for clinical application.

The Fc region of human immunoglobulin mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and plays an important role in the immune defense. ADCC refers to the phagocytosis or killing of target cells, which is mediated by the specific binding of antibody or the Fc fusion protein via the Fc region to the Fcγ receptor expressed on the surface of effector cells, such as natural killer cells and macrophages. CDC refers to the killing of target cells by the activation of complements, which is mediated by the specific binding of antibody or the Fc fusion protein via the Fc region to plasma proteins, such as complement proteins C1q, C3 and C4.

The effector function of the Fc fragment in rhG-CSF-Fc is reduced and eliminated by two methods (U.S. Patent: 6797493, Chinese Patent: 02126839.8). Among the four human IgGs, IgG2 has a weaker effector function. In regard to FcγRI and FcγRIIB, the affinity of FcγRI for the four human IgGs is in the order of: IgG1, IgG3 > IgG4 >> IgG2. The affinity of FcγRIIB for the four human IgGs is: IgG3 > IgG1 > IgG4 > IgG2 (Van Sorge et al 2003, Tissue Antigens. Vol. 61:189–202). Therefore, IgG2 Fc was used for the making of the fusion protein. Given the substitution of proline (a proven functional residue in CDC) at position 331 of IgG2 with serine, the fusion protein generated by this method no longer has any effector function. The above amino acid number (e.g. position 331) was calculated using the EU numbering system described by Kabat et al. (Sequence of Proteins of Immunological Interest, edition5, United States Department of Health and Human Services, 1991).

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. The sequence of this linker is: GlySerGlyGlyGlySerGlyGlyGlyGlySerGlyGlyGlyGlySer. The purpose is 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 also allow one G-CSF to be distant from another G-CSF, and produce rhG-CSF in a dimeric form with two G-CSF molecules on a single Fc fragment. The rhG-CSF-Fc dimer may cause a stronger receptor activation compared with a 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, 812-amino acid single-chain receptor with high affinity to G-CSF. During neutrophil development, each cell expresses about 50–500 G-CSFR. The more mature neutrophils are, the higher the density of G-CSFR on their surfaces Tamada et al. performed a 2.8A^{o} diffraction analysis of the G-CSF: G-CSFR crystal (PNAS, 2008, Vol. 103: 3135-3140) and found that this complex exists in a 2:2 ratio, i.e., 2 ligands with 2 receptors (Fig. 3-2). Each G-CSF molecule binds to one receptor. Only when two receptors binding to the 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). JAK2 then initiates gene transcription by activating STAT3, resulting in cell proliferation. Theoretically, dimeric ligands may be able to 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.

Furthermore, in a clinical study of rhG-CSF (Schwinger et al, 1993, Bone Marrow Transplantation, Vol. 11:489-492), it was found that peripheral blood precursor cells and peripheral blood stem cells increase drastically after the injection of rhG-CSF, which is called 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 the character of bone marrow matrix proteins and adhesion molecules have the similar function of PBPC and/or PBSC mobilization. Therefore, peripheral blood cells collected after rhG-CSF injection, are enriched with PBPCs and PBSCs, and are widely used as donor cells for conventional bone marrow transplantation in developed countries.

1.1.3 Preclinical pharmacokinetics study

Pharmacokinetics 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. Blood drug concentration and ANC increase (PD response) is dose-dependent. In the study of F-627 (100 μg/kg) subcutaneous injection in rats, $T_{1/2}$ was 7.6 ± 1.3 hours, C_{max} was 162 ng/mL, and AUC was 4217 ± 641 ng/mL·*hr. In the study of pegfilgrastim (Neulasta, 100 μg/kg) in rats, $T_{1/2}$ was 7.1 hours (Zamboni W.C, 2003, Pharmacotherapy, Vol.23 (8 pt2):9S-14S) and AUC was 1600 ng/mL·*hr (FDA IND). In cynomolgus monkey models with cyclophosphamide-induced neutropenia, the PK parameters of F-627 and pegfilgrastim at the equivalent dose were comparable (as shown in Table 30-1). However, F-627 resulted in a faster neutrophil recovery compared with pegfilgrastim.

Table 30-1. Comparison of PK parameters between F-627 and pegfilgrastim.

Dose 60 μg/kg	T_{max} (hr)	C_{max} (ng/mL)	AUC (ng/mL·hr)	$T_{½}$ (hr)
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 toxicology study

A systematic preclinical safety evaluation of F-627 has been conducted according to SFDA's registration categories of biological products and requirements for submission dossiers. Refer to Table 30-2 for the details of safety evaluation tests. In the repeat dosing toxicity studies in rats and cynomolgus monkeys, application requirements of FDA were taken into account by increasing the number of animals and the number of organs dissected. Meanwhile, the validation of PK/TK analysis method was in accordance with the application requirements of FDA/EMEA. 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.

Table 30-2. 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	Repeat dosing toxicity	75,225,675	Repeat	Administration:	675

Animal	Study Title	Dose (μg/kg)	Administration	Observation Time	NOAEL μg/kg
monkey	study			3 months Recovery period: 1 month	
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 for 5 times once every other day	30 minutes, 3 hours	
Rabbit	RBC in vitro hemolysis test	1000 μg/mL	In vitro incubation	3 hours	

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. F-627 is a recombinant fusion protein with a molecular weight of 95000 Daltons. Results from preclinical studies showed that the toxicity of F-627 was due to the exaggerated pharmacology effects, which is closely associated with the dose (expressed in mg/kg) in various different species. Based on the guideline issued by the FDA in 2005, the maximum safe starting dose of F-627 was calculated to be 67.5 μg/kg using mg/kg conversion, which is one-tenth of the NOAEL in cynomolgus monkeys. Based on the dose in the phase I clinical trial conducted in Australia, 80 μg/kg is a safe starting dose.

1.1.5 Preclinical pharmacodynamic 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 proliferation effects of F-627 on M-NFS-60 was 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 with filgrastim (Neupogen, Amgen) and pegfilgrastim (Neulasta, Amgen; Neulastim, Roche), 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 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-627 100 μg /kg in mice showed that, phosphorylated STAT3 levels in bone marrow cells increased 17 folds compared to baseline levels. Peak ANC in peripheral blood appeared at 48 hr after the dose, and recovered to normal levels at 72 hr. 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 cynomolgus monkey models with cyclophosphamide-induced neutropenia, F-627 not only shorten the recovery time of ANC, but also reduced the ANC decrease compared to filgrastim (rhG-CSF) and pegfilgrastim (Neulasta), thereby preventing the occurrence of severe neutropenia. F-627 demonstrated superior pharmacodynamic effects over the same dose (60 μg/kg) of pegfilgrastim.

1.1.6 Previous clinical studies

The phase I clinical trial was conducted at the Alfred Medical Center in Melbourne, Australia. The clinical study protocol was approved by the Ethics Committee of the hospital on May 4, 2010, and the first subject received F-627 treatment on Jun. 1, 2010.

The starting dose of this study was 30 μg/kg, and the dose was escalated sequentially to 60, 120, 240, and 360 μg/kg. In each dose cohort, 6 healthy adult male subjects were enrolled, which means totally 30 subjects in five cohorts. One to three Asian subjects were recruited to each dose cohort except for the 360 μg/kg cohort. Each subject received a single-dose of F-627 by subcutaneous injection. Blood samples were then collected at multiple time points in the 14-day follow-up visits to evaluate the pharmacokinetics, pharmacodynamics (including WBC, ANC, and CD34 analysis), and safety of the drug. The starting dose of F-627 was 30 μg/kg. Dose escalation was conducted only after it was confirmed to be safe by the principal investigators and medical experts of the sponsor.

All subjects completed dose administration. F-627 subcutaneous injection was well-tolerated. There were no serious adverse events (SAEs) or deaths among the 30 subjects. All adverse events are summarized in Table 30-3. No abnormalities were observed in the vital signs, electrocardiograms (ECGs), abdominal ultrasound, serum biomarkers and urinalysis parameters of subjects in all dose cohorts. The most common adverse events were manifested as musculoskeletal disorders, including mild to moderate muscle soreness, bone pain and lumbago.

Table 30-3. Summary of adverse events in the phase I clinical trial.

Adverse Events	30 μg/kg	60 μg/kg	120 μg/kg	240 μg/kg	360 μg/kg	Total Number	Description
Headache	1	2	1	1	2	7	2 moderate events
Injection-Site Pain	2			0		2	1 moderate events
Bone Pain		2		3		5	3 moderate events

Adverse Events	30 μg/kg	60 μg/kg	120 μg/kg	240 μg/kg	360 μg/kg	Total Number	Description
Back Pain	2	1	4	4	4	15	6 moderate events
Leg Pain		1		1	2	4	3 moderate events
Abdominal Discomfort/Pain		1		1		2	1 moderate events
Dry Skin		1				1	1 moderate events
Hunger			1			1	
Urinary Tract Infection				1		2	
Diarrhea				2		2	
Feeling Abnormal				1		1	
Tiredness					1	1	
Respiratory Tract Irritation				1		1	
Photophobia				1		1	

All moderate adverse events were noted, others were mild events.

Results are shown in Table 30-4. The 5 dose cohorts, 30, 60, 120, 240, and 360 μg/kg, demonstrated dose-dependent pharmacodynamics, i.e., WBC, ANC, and CD34 count increased with increasing dose. The lowest dose cohort (30 μg/kg) demonstrated significant ANC elevation. The time to initial onset of effect was 4 hours for all dose cohorts. In the lowest dose cohort, ANC at 4 hours after administration (5.05 ± 2.00) was significantly higher than that before administration (2.79 ± 0.40, p = 0.02). T_{max} of ANC was 36 hours for two low dose cohorts and 72 hours for two high dose cohorts. The peak ANC in these four dose cohorts increased 4.1, 4.4, 5.8, and 8.6 folds compared to the pre-dose ANC. CD34 peaked at 72–96 hours, respectively. CD34 reached 38.7 ± 11.7 in the 120 μg/kg cohort. Results showed a good dose-effect relationship of F-627 in healthy male subjects.

Table 30-4. Human PK parameters in phase I clinical trial.

Parameters	30 μg/kg (n=6)	60 μg/kg (n=6)	120 μg/kg (n=6)	240 μg/kg (n=6)	360 μg/kg (n=6)
C_{max} (ng/mL)	21.3(10.3)	44.6(17.7)	219.9(76.6)	759(160)	693(243)
T_{max} (hr)	8(8-16)	8(8-16)	16(16-36)	36(36)	16(16-48)
$T_{1/2}$ (hr)	43.9(9.3)	56.1(23.3)	59.3(23.5)	62.8(10.8)	71.4(27.3)
$AUC_{(0-\infty)}$ (ng·hr/mL)	720(214)	1756(673)	8374(2789)	46580(17255)	44009(18266)
CL/F (mL/hr/kg)	41.4(12.8)	36.8(14.6)	18.5(7.7)	5.7(2.0)	12.0/11.9

Figure 30-1. Semi-logarithmic plot of concentration-time (hours) after different doses of F-627 in healthy male subjects.

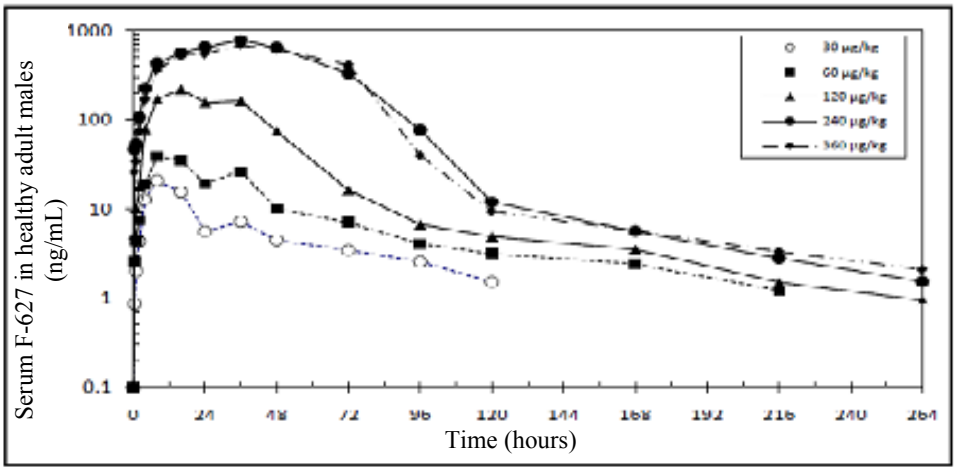
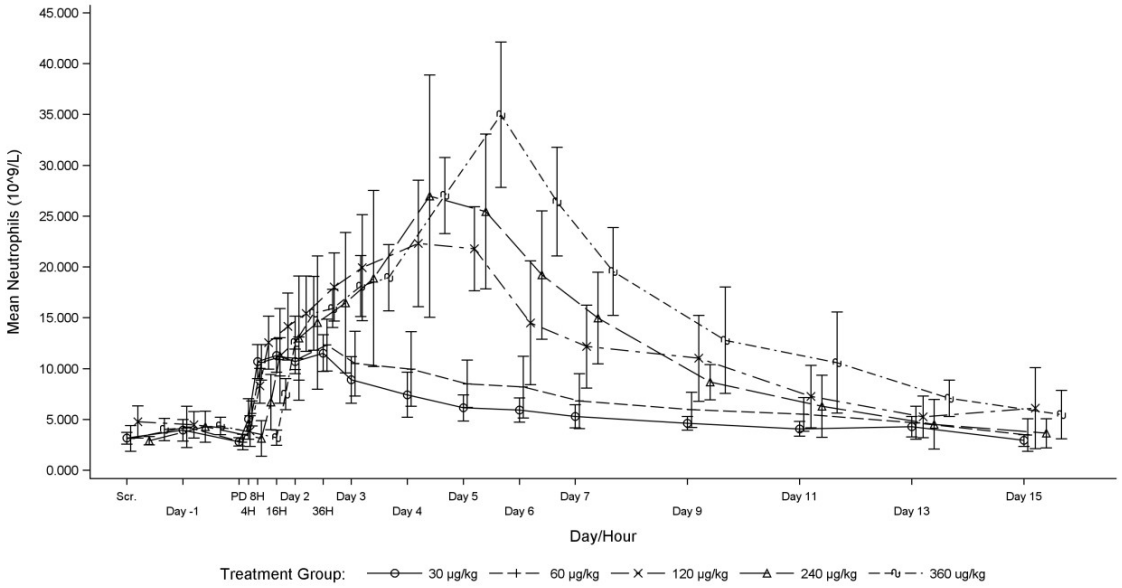


Table 30-5. Summary of pharmacodynamics in phase I clinical trial.

F-627 Dose μg/kg	ANC Elevation (T_{max}) hrs	Peak ANC (Increased Folds)	ANC Recovery hrs	CD34 T_{max} (CD34 Count)
30	4 (36)	11.52 (4.1)	144	96 (7.83 ± 2.32)
60	4 (36)	12.32 (4.4)	240	72 (21.5 ± 23.9)
120	4 (72)	22.31 (5.8)	288	72 (38.67 ± 11.71)
240	4 (72)	26.98 (8.6)	240	96 (37.83 ± 9.7)
360	5(96)	34.97(10.9)	To be supplemented	120(52.7 ± 19.0)

ANC units: 1×10^9/L; ANC recovery: ANC < 5×10^9/L; CD34 count units: 1×10^3/mL, n = 6.

Figure 30-2. Mean ANC in subjects after administration of different doses of F-627.



Clinical studies of long-acting rhG-CSF (pegfilgrastim, i.e. PEGylated G-CSF) were primarily conducted by Amgen Inc. The new drug approval documents of pegfilgrastim included two phase I clinical trials that examined the PK/PD and safety of the drug in healthy human; four phase II clinical trials that determined the effective dose of the drug in patients with different cancers, including post-chemotherapy lymphoma, lung cancer and breast cancer; and two phase III clinical trials that examined the efficacy and safety of 100 μg/kg pegfilgrastim. Pegfilgrastim was approved for marketing by the FDA in 2002 and is mainly used in Europe, U.S. and other western countries. Pegfilgrastim is a first-line supportive therapy for post-chemotherapy cancer patients. It is used to treat neutropenia caused by radiation therapy and chemotherapy.

There has been a substantial number of clinical studies of pegfilgrastim in recent years. A total of 107 clinical studies could be found in the Pubmed database using pegfilgrastim or Neulasta as keywords for title and abstract. Long-acting pegfilgrastim is favorable for patients as it is administered once per chemotherapy cycle and its efficacy is equal to that of the short-acting Filgrastim administered daily. However, most blood cancer patients, such as those with lymphoma or acute leukemia, are in a state of severe neutropenia for a considerable period of time (2-3 weeks) even with post-chemotherapy administration of filgrastim or pegfilgrastim. Therefore, this is the medical challenge facing the application of the existing recombinant human G-CSF monomers.

The clinical side effects of long-acting Pegfilgrastim are similar with those of short-acting Filgrastim. These side effects are primarily manifested as musculoskeletal disorders including muscle soreness, bone pain, lumbago and chest pain, and gastrointestinal disorders including inappetence or increased ALT/AST in liver. Some patients may develop fever, headache, asthenia, rash and ALP/LDH elevation, and a small fraction of patients may experience shock, interstitial pneumonia, adult respiratory distress syndrome, and increased immature granulocyte count. Pegfilgrastim is prohibited for individuals with severe liver, kidney, heart and lung disorders. Pegfilgrastim is not recommended for myeloid leukemia patients without significant reduction in immature granulocytes in the bone marrow or with immature granulocytes in the peripheral blood.

1.2 Overall Evaluation of Adjuvant Measures and Efficacy of Chemotherapy for Cancer

Cancer is currently still a threat to human health and its incidence rate continues to increase (Nature Review Cancer, 2006, vol 6: 63-74). In 2002, there were 10.9 million new cancer patients worldwide. The number of new cancer patients worldwide is estimated to reach 16.5 million in 2020 and 27.0 million in 2050. According to the statistics from the Ministry of Health of the PRC, the incidence rate of cancer had increased to 127 in 100000 people in the 1990s. In recent years, the number of new cancer patients per year has reached 1.6–1.7 million, totaling about 4.5 million cancer patients. In terms of cancer treatment, chemotherapeutic and cytotoxic agents are still first-line therapy. Some new small molecule targeted drugs still cannot be used as first-line drugs for curing cancer. Therefore, there is still much to be done for the adjuvant therapy of chemotherapy. Although first- and second-generation G-CSF has partially shortened the duration of chemotherapy-induced neutropenia, how to alleviate neutropenia and shorten the duration of severe neutropenia are still the challenges in the treatment of chemotherapy-induced severe neutropenia. The development and study of F-627 have provided theoretical and practical feasibility for the treatment of this problem. As an independent global innovation, F-627 is expected to become the best adjuvant therapy for enhancing the efficacy of chemotherapy drugs in cancer patients.

2. STUDY OBJECTIVES

2.1 Primary Objective

To evaluate the safety and tolerability of recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection (F-627) in treatment of female postoperative breast cancer patients who require adjuvant chemotherapy in a single-center, open-label, single-dose and repeated-dose, dose-escalation phase I clinical trial.

2.2 Secondary Objectives

1) To evaluate the pharmacokinetics (PK) of F-627 by determining the serum drug concentrations of F-627 at different time points after dosing;
2) To evaluate the pharmacodynamics of F-627 by analyzing the relationship between serum drug concentrations of F-627 and neutrophil counts in blood at different time points after dosing, and to provide a recommended dose for phase II clinical trial.

2.3 Exploratory Objectives

1) To observe the number of days in which absolute neutrophil count is less than 0.5×10^9/L in cycle 1 of chemotherapy, the average time of ANC recovery, and the number of days in which absolute neutrophil count is less than 1×10^9/L, and to provide insights for phase II clinical trial.
2) To evaluate the potential immunogenicity of F-627 by testing anti-F-627 antibodies (IgG and IgM) in serum.

3. STUDY PLAN AND METHODOLOGY

3.1 Overall Study Design and Schedule of Study Procedures

This is a single-center, open-label, dose-escalation, single-dose and repeated-dose phase I clinical trial in treatment of female postoperative breast cancer patients who require adjuvant chemotherapy. Eligible patients are those who have not received chemotherapy. The adjuvant chemotherapy regimen for this trial is EC→P or EC→T, 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 all 4 cycles, subjects will receive 4 subsequent cycles of sequential chemotherapy (either paclitaxel or docetaxel) and supportive care according to routine clinical practice. The investigator must ensure that the first cycle of treatment follows the recommended chemotherapy regimen; in chemotherapy cycles 2–4, treatment regimen and dose are permitted to be individualized based on subject conditions. Dose delay and one dose reduction due to toxicities other than myelotoxicity (such as cardiotoxicity) are permitted in this trial. If the subject requires a second dose reduction, the investigator and sponsor must decide together whether the subject should continue the treatment with study drug.

Subjects who have signed the informed consent form and have passed the screening are enrolled in the trial.

This study includes 3 dose cohorts, 80, 240, and 320 μg/kg, each of which will enroll 6 subjects sequentially. Subjects in each cohort will receive a single-dose of F-627 by subcutaneous injection approximately 48 hours after the completion of chemotherapy. Blood samples are then collected at multiple time points in the subsequent follow-up visits to evaluate the pharmacokinetics, pharmacodynamics, and safety of the drug. If dose-limiting toxicities (DLT, see Section 3.4) are not observed before the start of cycle 2, then the same dose of F-627 is to be given at approximately 48 hours after each chemotherapy in cycles 2–4.

Only after 6 subjects in each cohort have completed the treatment and observation of the first cycle will the sponsor and investigator determine whether to proceed to the next higher dose based on the safety evaluation.

Schedule of study procedures is detailed in Table 30-6.

Table 30-6. Schedule of F-627 study procedures.

	1–15 Days Before Enrollment	Day 1, Day 22, Day 43, and Day 64 of the Study (Window Phase: 3 Days Before Dosing)[6]	Day 3, Day 24, Day 45, and Day 66 of the Study [1]	Cycle 1 of Chemotherapy (Day 3–Day 21)[4]	Cycles 2–4 of Chemotherapy (Day 3–Day 21)[5]	End of Study (Day 84)[7]
Signing Informed Consent Form	X					
History of Cancer	X					
Complete Physical Examination	X					X
Physical Examination		X	X			
Abdominal Ultrasound[2]	X	X				X
Chemotherapy		X				
Urinalysis[2]	X	X				X
Administration[1]			X			
12-Lead ECG[3]	X					X
Color Doppler Echocardiography	X					
Chest X-Ray	X					X
Height and Weight[2]	X	X				
Body Temperature	X	X	X	X	X	X
Routine Blood Test (Including ANC)	X	X	X	X	X	X
Clinical Chemistry[2]	X	X				X
Serum Pregnancy Test	X					X
Blood Pressure and Heart Rate	X	X				X

	1–15 Days Before Enrollment	Day 1, Day 22, Day 43, and Day 64 of the Study (Window Phase: 3 Days Before Dosing)[6]	Day 3, Day 24, Day 45, and Day 66 of the Study [1]	Cycle 1 of Chemotherapy (Day 3–Day 21)[4]	Cycles 2–4 of Chemotherapy (Day 3–Day 21)[5]	End of Study (Day 84)[7]
Pharmacokinetics[8]			X	X	X	
Serum Antibody[9]		X	X	X	X	X
AEs and Combined Medications[9]		X	X	X	X	X

1. Three dose cohorts for F-627 (80 μg/kg, 240 μg/kg and 320 μg/kg);
2. Examination should be completed upon enrollment and the start of each chemotherapy cycle. Body height is measured upon enrollment only, while body weight is measured on day 1 of each cycle; for abdominal ultrasound, a retest on day 1 of cycle 1 is not required and the baseline result can be used; for routine blood test and clinical chemistry before the start of cycle 1, results within 7 days are acceptable;
3. 12-lead ECG should be repeated at the last visit of the study;
4. Starting on day 3 of cycle 1, oral temperature measurement and routine blood test will be performed daily until ANC recovers from its nadir to > 1.0×10^9/L, and once every 3 days thereafter until the next cycle;
5. For chemotherapy cycles 2–4 (day 3–day 21 of each chemotherapy cycle, i.e., day 24–day 84 of the study), starting on each day 3 of cycles 2–4, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to > 1.0×10^9/L, and once every 3 days thereafter until the next cycle;
6. Only when ANC of a subject recovers to > 1.0×10^9/L as judged by the investigator, can the next cycle start;
7. The last visit is on day 84, and the subjects should complete a follow-up visit by telephone on day 114 (30 days after the last visit);
8. In cycle 1 and cycle 3, blood samples are collected for PK at time points shown in Table 30-8;
9. Blood samples will be collected for serum antibody assays on days 1, 8, and 13 of each cycle as well as day 22 of cycle 4.

Remark: All laboratory measurements will be performed at a central laboratory assigned by the investigator. In case of any toxicities related to chemotherapy, subsequent treatment can be modified according to common diagnosis and treatment practice in the judgment of the investigator.

3.2 Evidence for Dose Determination

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. F-627 is a recombinant fusion protein with a molecular weight of 95000 Daltons. Results from preclinical studies showed that the toxicity of F-627 was due to the amplification of pharmacodynamics, which is closely associated with the dose (expressed in mg/kg) in various different species. Based on the guideline issued by the FDA in 2005, the maximum safe starting dose of F-627 was calculated to be 67.5 μg/kg using mg/kg conversion, which is one-tenth of the NOAEL in cynomolgus monkeys. Based on the dose for healthy volunteers in the phase I clinical trial, 80 μg/kg is a safe starting dose.

The pharmacokinetic parameters in cynomolgus monkeys, such as $T_{1/2}$, AUC, CL and MRT, were comparable between F-627 and pegfilgrastim. In the 3-month repeat dosing toxicity studies of pegfilgrastim and F-627 in rats and cynomolgus monkeys based on subcutaneous injection, the no-observed-adverse-effect-levels (NOAELs) of drugs in rats were comparable to those in cynomolgus monkeys. In addition, the phase I clinical trial involving healthy volunteers used an F-627 dose that is less than 5% of the NOAEL in monkeys (Table 30-6). F-627 demonstrated good safety and tolerability in all five dose cohorts (30, 60, 120, 240 and 360 μg/kg). For F-627, 240 μg/kg was equivalent to 96 μg/kg of pegfilgrastim (calculated by the molarity of G-CSF). The phase I of this study plans to

include three dose cohorts, namely 80, 240 and 320 μg/kg. Firstly, all doses should not exceed the maximum dose in the phase I clinical trial. Secondly, the G-CSF molarity of 240 μg/kg F-627 is comparable to that of 100 μg/kg pegfilgrastim. Finally, 60 μg/kg of F-627 showed pharmacodynamics superior than 150 μg/kg in the cynomolgus monkey models of chemotherapy-induced neutropenia. Therefore, using 80 μg/kg in the low-dose cohort will ensure that the drug may be effective for the subjects. The highest dose (320 μg/kg) for subjects is slightly lower than the maximum dose (360 μg/kg) tested in healthy subjects.

Table 30-7. Rationale for F-627 doses in clinical trials.

	Pegfilgrastim	F-627
Repeat Dosing Toxicity Study in Rats	100, 300, and 1000μg/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
Starting Dose of Healthy Subjects in Phase I Clinical Trial	30 μg/kg	30 μg/kg
Dose of Healthy Subjects in Phase I Clinical Trial	30, 60, 100, 300 μg/kg	30, 60, 120, 240, 360 μg/kg
Post-Chemotherapy Dose for Breast Cancer in Clinical Trial	60, 100, 300 μg/kg	Planned: 80, 240 and 320 μg/kg
Recommended Dose for Clinical Trials	100 μg/kg	TBD

3.3 Single Dose and Repeated Dose

This study includes 3 dose cohorts, 80, 240, and 320 μg/kg, each of which will enroll 6 patients with breast cancer sequentially receiving adjuvant chemotherapy. Subjects in each cohort will receive a single-dose of F-627 by subcutaneous injection approximately 48 hours after the completion of chemotherapy. Blood samples are then collected at multiple time points in the subsequent follow-up visits to evaluate the pharmacokinetics, pharmacodynamics, and safety of the drug. If dose-limiting toxicities (DLT, see Section 3.4) are not observed before the start of cycle 2, then the same dose of F-627 is to be given at approximately 48 hours after each chemotherapy in cycles 2–4.

3.4 Dose Escalation

Doses that will be used in this study are 80, 240 and 320 μg/kg, and 6 subjects will be enrolled in each dose cohort. The starting dose is 80 μg/kg. Only after 6 subjects have completed the treatment and observation of the first cycle will the sponsor and investigator determine whether to proceed to the next higher dose based on the safety evaluation. Likewise, the 240 μg/kg cohort will enroll 6 subjects, and only after 6 subjects have completed the treatment and observation of the first cycle will the sponsor and investigator determine whether to proceed to the next higher dose based on the safety evaluation. The 320 μg/kg cohort will enroll 6 subjects, but dose escalation will not be conducted after all 6 subjects have completed the treatment and observation.

Dose Cohort	Dose	Number of Subjects
1	80 μg/kg	6
2	240 μg/kg	6
3	320 μg/kg	6

Dose escalation should be stopped in each dose cohort if 2 dose-limiting toxicities (DLT, see Section 3.5 for definition) are observed in subjects in cycle 1 of chemotherapy

3.5 Dose-Limiting Toxicity (DLT)

Dose-limiting toxicity (DLT) refers to an intolerable toxicity that is observed in the treatment with investigational drug and limits the further dose escalations. DLT is defined as any grade 3 or greater adverse event related to the investigational drug that observed in cycle 1 (21 days). Adverse events will be assessed according to NCI CTCAE V4.03 criteria.

After 6 subjects in each F-627 dose cohort have completed the treatment and observation of first cycle, the trial may proceed into the next dose cohort only if less than 2 subjects develop DLT. If a grade 3 or greater adverse event related to the investigational drug is observed in cycles 2–4, then the investigator and sponsor will determine together whether the adverse event would affect further dose escalations.

3.6 Risk, Benefit and Ethical Evaluations

The rhG-CSF-Fc fusion protein is a long-acting rhG-CSF expressed in CHO cells. Each molecule of rhG-CSF-Fc fusion protein contains two G-CSF dimer molecules, which may overcome the weak bioactivity of pegfilgrastim and produce a stronger receptor activation signal, thereby accelerating the differentiation and proliferation of neutrophils in bone marrow. Also, preclinical studies have shown that the pharmacokinetic and pharmacodynamic properties of rhG-CSF-Fc is different from or superior to pegfilgrastim, and therefore it decreases the severity of neutropenia and reduces the duration of severe neutropenia in cancer patients after chemotherapy. For the phase I clinical trial, patients with breast cancer will be enrolled as subjects to evaluate the safety, tolerability and pharmacodynamic properties of F-627. Phase I clinical trial of pegfilgrastim showed that subcutaneous injection of 30, 60, 100 and 300 μg/kg of pegfilgrastim resulted in no significant toxicity. The dose of F-627 will also be controlled within this range to ensure the safety of the subjects. Phase I clinical trial of F-627 in Australia already demonstrated that no serious adverse event is observed within this dose range and subjects had good treatment compliance.

In this study, the safety of the subjects will be closely monitored, including clinical symptoms, vital signs, routine blood test, clinical chemistry, urinalysis and any adverse event. Once an adverse event is observed, it will be handled in accordance with the national regulations. Safety evaluation of the phase I clinical trial on healthy subjects showed that adverse events observed under the above doses were all within controllable range. Emergency medical events and handling procedures are described in Section 11 of this document.

4. STUDY POPULATION

4.1 Study Population and Number of Subjects

Female postoperative patients with breast cancer who receive adjuvant chemotherapy will be enrolled in this study. Eligible subject should be chemotherapy naïve patient.

The selection of chemotherapy drug is one of the key considerations in the design of this protocol. Among the adjuvant chemotherapy regimens that are recommended by the NCCN 2010 guideline for breast cancer, the preferred regimens include TAC, dose-dense AC → dose-dense paclitaxel, AC → paclitaxel, TC, EC, and AC. At present, AC/EC→P or AC/EC→T are the commonly used regimens in China.

The adjuvant chemotherapy regimen for this trial is EC→P or EC→T, that is: 100 mg/m^2 epirubicin i.v. + 600 mg/m^2 cyclophosphamide i.v. on day 1, repeat cycle every 21 days for 4 cycles. After completing the evaluation for all 4 cycles, subjects will receive 4 subsequent cycles of sequential chemotherapy (either paclitaxel or docetaxel) and supportive care according to routine clinical practice.

The investigator must ensure that the first cycle of treatment follows the recommended chemotherapy regimen; in chemotherapy cycles 2–4, treatment regimen and dose are permitted to be individualized based on subject conditions. Dose delay and one dose reduction due to chemotherapy-induced toxicities (such as cardiotoxicity) are permitted in this trial. If the subject requires a second dose reduction, the investigator and sponsor must decide together whether the subject should continue the treatment with study drug.

This study includes 3 dose cohorts, 80, 240, and 320 μg/kg, each of which will enroll 6 subjects, totaling 18 subjects.

4.2 Inclusion Criteria

1) 18-75 years old;
2) Female postoperative breast cancer patients who require adjuvant chemotherapy, and are planned to receive 4 cycles of EC chemotherapy;
3) ECOG performance status of 0-1;
4) Absolute neutrophil count (ANC) ≥ 2.0 × 10^9/L, hemoglobin (Hb) ≥ 11.0 g/dL, and platelet (PLT) ≥ 100 × 10^9/L prior to chemotherapy;
5) Hepatic and renal function within the normal range;
6) Left ventricular ejection fraction greater than 50%
7) Willing to sign the informed consent form and able to comply with protocol requirements;

4.3 Exclusion Criteria

Subjects who meet any of the following must be excluded from this study:

1) Women in pregnancy or breastfeeding; women of child–bearing potential have a positive pregnancy test result prior to the first dose;
2) Life expectancy less than 12 months;
3) Radiation therapy within 4 weeks prior to enrollment;
4) Patients with breast cancer who have received neoadjuvant chemotherapy before radical mastectomy;
5) Prior bone marrow or stem cell transplant;
6) With other malignant tumors other than breast cancer;
7) Have received G-CSF treatment within 6 weeks prior to enrollment;
8) Diagnosed with acute congestive heart failure, cardiomyopathy, or myocardial infarction by clinical diagnosis, ECG or other approaches
9) With any disease that may cause splenomegaly;
10) With acute infection, chronic active Hepatitis B within 1 year (unless patients tested negative for HBsAg prior to enrollment) or Hepatitis C;
11) With active tuberculosis (TB); history of TB exposure, unless negative for tuberculin test; or TB patients undergoing treatment; or suspected TB evaluated by chest x-ray;
12) Known HIV positive or AIDS;
13) With sickle cell anemia;
14) With alcohol or drug abuse that may affect the compliance with the study;
15) With known hypersensitivity to E. coli derived proteins, G-CSF, or excipients;
16) Has received any other study drug within 4 weeks prior to enrollment;
17) Patients with diseases or symptoms unsuitable for participating in the trial based on the investigator's judgment;

4.4 Criteria for Terminating Study

1) Incidence rate and severity of serious adverse events (SAEs) indicate the study should be terminated early as determined by the investigators and the sponsor;
2) The dose-limiting toxicity of the subjects does not recover or cannot be relieved;
3) The investigators question the safety of the drug and believe that the continuation of the study may pose serious risks to the subjects;
4) The maximum dose set in the clinical trial has been reached;
5) Data fraud, or inaccurate/incomplete collection of data;

4.5 Criteria for Subject Withdrawal

Subjects will prematurely withdraw from the study with a written explanation when the following conditions occur.

1) Subjects voluntarily withdraw during the study;
2) The investigator considers that withdrawal is for the best interest of the subject;
3) The investigator or subject believes that continuing the trial may result in intolerable adverse events;
4) Complications or worsening co-morbidities affecting subject's participation occur;
5) Safety issues of the study drug in the absence of data, subject will be exposed to potential risks if continues the participation in the study;
6) When a safety concern regarding the study drug arises, but data is yet unknown, resulting in potential risks if subjects continue the trial;

4.6 Drop-Out Criteria

Determination of drop-outs: Eligible subjects who have signed the informed consent form and been enrolled have the right to withdraw from the study at any time. Subjects who do not complete the entire observation are considered drop-outs regardless of the time or reason of the withdrawal. A replacement should be implemented immediately according to the original plan when a drop-out occurs. Blood samples from drop-out subjects should be retained and tested and processed by the sponsor.

4.7 Withdrawal-Related Procedures and Handling

The investigator should ask the withdrawn subject for the reason of withdrawal and whether there is an adverse event, and record the length of treatment and dose. If possible, the investigator should perform corresponding observation and evaluation of the withdrawn subject and conduct a follow-up for adverse events within 30 days of the last dosing. If a subject withdraws from the study due to suspected grade 2, 3 or 4 infection according to WHO risk classification criteria, then his/her biological samples must not be sent to a laboratory, and instead, should be destructed as per operating practice of the study site.

5. INVESTIGATIONAL DRUG AND TREATMENT

5.1 Source

F-627 is provided by Generon (Shanghai) Corporation Ltd.

5.2 Strength and Expiration Date

The recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection (F-627) is securely packaged and sealed by Generon (Shanghai) Corporation Ltd.

Dosage Form	Strength	Formulation		Mode of Administration	Expiration Date
Lyophilized Powder for Injection	1 mg	F-627 (rhG-CSF-Fc) Sucrose Mannitol Polysorbate 80 Sodium phosphate dibasic Sodium phosphate monobasic	1.0 mg 20.0 mg 50.0 mg 0.05 mg 0.92 mg 0.42 mg	Subcutaneous injection	2 years (tentative)
Lyophilized Powder for Injection	5 mg	F-627 (rhG-CSF-Fc) Sucrose Mannitol Polysorbate 80 Sodium phosphate dibasic Sodium phosphate monobasic	5.0 mg 20.0 mg 50.0 mg 0.05 mg 0.92 mg 0.42 mg	Subcutaneous injection	2 years (tentative)

5.3 Labeling

The recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection (F-627) is stored as lyophilized powder in tubular vials (1.0 mg/2 mL vial and 5.0 mg/2 mL vial). The drug label includes the following information: investigational drug name, study number, study protocol number, storage conditions, contents, mode of administration, batch number, expiration date, and the standard notes "For clinical trials only" and "Keep out of reach of children".

5.4 Storage

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.

5.5 Drug Preparation

The pharmacist is responsible for preparing the drugs and the nurse is responsible for reviewing. During drug preparation, inject 0.5 mL of water for injection slowly into the vial along the wall, let the vial stand for 1–2 minutes, and perform subcutaneous injection after the powder is completely dissolved. The injection should be used within 1 hour after preparation. See the drug preparation procedure manual. The drug is generally injected in the morning (09:00 ± 1:00). The subject should remain in a sitting or walking position within two hours after the injection.

5.6 Administration and Precautions

Subjects must sign the informed consent forms prior to enrollment. After being screened and confirmed to be enrolled, subjects will be numbered in strict order. If a subject withdraws from the study, the same subject number must not be reused. Subjects who have withdrawn are not allowed to participate in this study again. According to the study protocol, subjects are allocated to different dose cohorts and will enter the study in the order of enrollment.

The investigator should be present during administration and within 1 hour after administration to provide guidance and supervision, and be prepared to administer first aid or emergency treatment whenever needed. The specific time and date of dosing, and the drug labels should be documented in the subject's source data files and case report forms.

Subjects will receive a chemotherapy of EC on day 1 of each cycle, and a subcutaneous injection of F-627 on day 3, that is, 48 ± 2 hours after the start of chemotherapy. During the treatment, subjects should avoid strenuous physical activity or long periods of bed rest, and keep relaxed.

5.7 Salvage Therapy

In the study, an approved G-CSF treatment should be given to the patient as salvage therapy in the case of febrile neutropenia (defined as ANC < 1.0×10^9/L; a single measurement of body temperature > 38.3 °C or a fever ≥ 38.0 °C lasting for longer than 1 hour; note: body temperature measurement is based on oral temperature or equivalent armpit/rectal temperature) or grade 4 neutropenia for longer than 3 days. The sponsor recommends GRAN® (filgrastim) at a dose of 5 μg/kg/day of G-CSF, once daily for ≤ 2 weeks or until neutrophil count recovers to 1.0×10^9/L.

5.8 Drug Management

Registration and recording of the investigational drug should be in the charge of designated personnel. The study site should establish a set of systematic operating procedures to ensure that the drugs are received by designated personnel, the distribution of the drugs is accurately documented, and the drugs are used and stored properly.

1) Drug registration form:
   a. The type of drug received from the sponsor;
   b. Dosage form and strength;
   c. Batch number and expiration date;
   d. The dosage form, dose, quantity, and date of drug dispensed to the investigator (signed by both dispensers and recipients).
2) Individual drug administration record: subject name, package number, administration time, dose and mode of administration, signature of operator, and return of packaging.
3) Detailed record of drug administration, loss, scattering, and misuse.

4) Unused drugs should be stored in accordance with storage requirements. Storage conditions should be checked regularly by the same designated personnel.
5) After the study is completed, all remaining drugs should be returned and recorded, and be disposed according to the GCP by the site together with the sponsor.

5.9 Principles and Methods for Treatment of Drug Toxicities

If a subject develops a mild drug toxicity after receiving F-627 injection, symptoms should be monitored with the treatment continued. Intervention is usually not required. Toxicities related to chemotherapy should be handled according to clinical guidelines and the prescribing information of the chemotherapy drugs. Serious or life-threatening symptoms must be treated immediately, and the clinical trial for this case must be terminated.

Refer to Table 30-3 for all adverse events observed in phase I clinical trial: these symptoms do not need to be treated. At present, serious adverse events have not been observed for F-627. High-risk serious adverse events for long-acting G-CSF that have been reported include:

- Acute splenomegaly: incidence rate is around 1–10%, while the likelihood of splenic rupture is less than 1 in 10000. Patients who develop abdominal pain and were found to have splenomegaly upon physical examination or ultrasonic inspection should be treated immediately. The drugs should be discontinued.
- Adult respiratory distress syndrome (ARDS): Rare cases have been reported with clinical use of long-acting G-CSF. Treat immediately and discontinue the drugs if present.

5.10 Treatment Compliance

Administer according to subject's dosage allocation under the guidance and supervision of the investigator. The actual time and date of administration should be accurately documented for each subject. Any protocol violations should be documented in subject's source data file and case report form.

6. EVALUATIONS AND ENDPOINTS

6.1 Evaluations During Screening

Prior to enrollment, the evaluation carried out within 2 weeks before dose administration (1–14 days before enrollment) includes:

1) Record of subject demographics: date of birth, gender, ethnicity, etc.;
2) Medical history collection, including history of cancer and history of chemotherapy;
3) Record of concomitant diseases and combined medication, surgical history, etc.;
4) Record of pathological diagnosis confirmed in tumor histology or cytology;

5) Tumor staging (TNM);
   - ✧ Complete physical examination, height, weight, vital signs (temperature, pulse, respiratory rate, blood pressure), and heart rate;
6) ECOG PS score, weight evaluation;
7) Hematologic examination: 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.;
8) Clinical chemistry: including total protein (TP), albumin (ALB), globulin (G), blood glucose, blood urea nitrogen, creatinine, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), pancreatic amylase, total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), Ca, P, Mg, K, Na, Cl, etc.;
9) Urinalysis: including pH, specific gravity, protein, cast, blood cell, urine glucose, ketones, etc.;
10) 12-lead ECG;
11) Color doppler echocardiography
12) Pregnancy test within 7 days before dose administration;
13) Chest x-ray (PA and lateral);
14) Signing informed consent form;
15) Abdominal ultrasound (liver, gallbladder, spleen, kidney, and pancreas)

Note: Only results of routine blood test and clinical chemistry conducted within 1 week before enrollment are accepted.

6.2 Clinical Evaluations During and After F-627 Treatment

Evaluations on day 1 of each chemotherapy cycle (day 1, day 22, day 43, and day 64 of the study) include:

1) Abdominal ultrasound;
2) Chemotherapy;
3) Urinalysis;
4) Height, weight;
5) Body temperature;
6) Routine blood test (including ANC)
7) Clinical chemistry;
8) Blood pressure and heart rate;
9) Serum antibody assay (IgG and IgM)
10) Adverse events and combined medication;

Evaluations on day 3 of each chemotherapy cycle (day 3, day 24, day 45, and day 66 of the study) include:

1) Physical examination
2) Investigational Drug;
3) Temperature;
4) Routine blood test (including ANC);
5) Adverse events and combined medication;

According to different time in the chemotherapy cycle, for cycle 1 (days 1–21 of both the cycle and the study), starting from day 3, oral temperature measurement and routine blood test will be performed daily until ANC recovers from nadir to $> 1.0 \times 10^9$/L, and once every 3 days thereafter until the next cycle. Evaluations include:

1) Temperature;
2) Routine blood test (including ANC);
3) Adverse events and combined medication;

For chemotherapy cycles 2–4 (days 3–21 of each chemotherapy cycle, i.e., days 23–84 of the study), starting from day 3, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to $> 1.0 \times 10^9$/L, and once every 3 days thereafter until the next cycle. Evaluations include:

1) Physical examination;
2) Temperature;
3) Routine blood test (including ANC);
4) Serum antibody assay (IgG and IgM);
5) Adverse events and combined medication

Evaluations upon the end (day 84) or withdrawal include:

1) Physical examination;
2) Abdominal ultrasound;
3) Urinalysis;
4) 12-lead ECG;
5) Chest x-ray;
6) Temperature;
7) Routine blood test (including ANC)
8) Clinical chemistry;
9) Serum pregnancy test;
10) Blood pressure and heart rate;

11) Antibody test (IgG and IgM);
12) Adverse events and combined medication

Subjects who withdraw from the study should be followed for adverse events 30 days after the last dose.

The subjects should complete telephone visits (on day 114, i.e., 30 days after the last visits).

6.3 Pharmacokinetic (PK) Evaluations

6.3.1 Determination and analysis of PK serum samples

The concentrations of drugs and metabolites in blood samples will be determined by the validated central laboratory (Covance Pharmaceutical Research and Development (Shanghai) Co., Ltd.). The specific method of determining serum drug concentrations will be decided by the laboratory and will be described in detail in the clinical study report.

6.3.2 Collection, handling, and storage of PK blood samples

According to the time points specified in the study protocol, baseline blood samples are collected within 1 hour before dose administration. Refer to 30-8 for the blood collection interval. The actual blood collection time should be documented after each intravenous blood sampling. The sampling date and collection tube number should be documented in the case report form (CRF). The collect tube label should include the following information: study number, subject initials, cycle number, day, sampling time (hour), etc. Each time a total of 2.5 mL of blood is collected, transferred to a labeled serum separator tube, placed for about 30 minutes, and centrifuged at 1000 g for 15 minutes at room temperature. Aliquot the serum into two clean 1.5 mL EP tubes (approximately 0.3 mL of serum each), close the lid tightly and immediately transfer to ≤ –70 °C for storage. The entire sampling and serum collection process must be completed within 60 minutes. Samples must be transferred in a constant temperature box with dry ice.

6.3.3 Blood sampling of single-dose and repeated-dose PK studies

PK analysis of F-627 in the phase I clinical trial showed that mean time to peak was 30–36 hours and plasma elimination half-life was 46–72 hours, longer in the high-dose cohort. For example, in the 240 μg/kg cohort, T_{max} was 36 hours, $T_{1/2}$ was 62.8 hours, MRT was 43.5 ± 6.0 hours, C_{max} was 758 ± 160 ng/mL, AUC was 46580 ± 17255 ng/mL*hr, and CL_z/F was 5.72 ± 2.0 mL/hr/kg.

Taking into account the above results from animal PK studies, this phase I clinical trial plans to collect blood samples at different time points following single-dose and repeated-dose administration. See Table 30-8 for detailed blood sampling time.

Table 30-8. Blood sampling time of single-dose and repeated-dose PK studies.

Study Process	Days of Study	Days of Dosing	Blood Sampling Time Point of Cycle 1 (hours: minutes)	Blood Sample Number	Blood Sampling Time Point of Cycle 3 (hours: minutes)	Blood Sample Number
				F-627 PK Study (2.5 mL)		F-627 PK Study (2.5 mL)
Study Process	**3**	1	–01:00±0.1:00	PK 1	00:00±0.1:00	PK 1
			02:00±0.3:00	PK 2	02:00±0.3:00	PK 2
			06:00±0.5:00	PK 3	06:00±0.5:00	PK 3
			12:00±0.5:00	PK 4	12.00±0.5:00	PK 4
	4	2	24:00±0.5:00	PK 5	24:00±0.5:00	PK 5
			36:00±0.5:00	PK 6	36:00±0.5:00	PK 6
	5	3	48:00±1:00	PK 7	48:00±1:00	PK 7
	6	4	72:00±1:00	PK 8	72:00±1:00	PK 8
	7	5	96:00±1:00	PK 9	96:00±1:00	PK 9
	8	6	120:00±1:00	PK 10	120:00±1:00	PK 10
	9	7	144:00±1:00	PK 11	144:00±1:00	PK 11
	11	9	192:00±2:00	PK 12	192:00±2:00	PK 12
	13	11	240:00±2:00	PK 13	240:00±2:00	PK 13
	16	14	312:00±2 4:00	PK 14	312:00±24:00	PK 14
	21	19	432:00±2 4:00	PK 15	432:00±24:00	PK 15
Number of Blood Samples Collected on Each Subject				15		15
Total Volume of Blood Samples Collected on Each Subject				37.5mL		37.5 mL

6.4 Pharmacodynamic Evaluation

While evaluating the safety, tolerability and pharmacokinetic characteristics of F-627, the ANC-time profile after treatment is observed to investigate the effects of different dosages on neutrophil count and to determine a recommended dose for phase II trials.

Pharmacodynamic evaluation of F-627 is carried out at the study site. For cycle 1, starting from day 3, oral temperature measurement and routine blood test will be performed daily until ANC recovers from its nadir to a value not less than 10×10^9/L, and once every 3 days thereafter until the next cycle; for chemotherapy cycles 2–4 (days 3–21 of each chemotherapy cycle, i.e., days 24–84 of the study), starting from each day 3 of cycles 2–4, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to $> 10 \times 10^9$/L, and once every 3 days thereafter until the next cycle. Refer to Table 30-6 for specific procedures.

6.5 Safety Evaluation

Safety evaluations include all observed and recorded adverse events and serious adverse events, changes in complete physical examinations, vital signs, performance status, routine blood test, routine urinalysis, chemistry indicators, echocardiogram, and ECG.

Refer to Section 7 for the definition of adverse events, which are graded according to NCI CTCAE V4.03. The investigator should take appropriate treatment measures on the adverse events, report the event in the corresponding form of CRFs, and determine the relationship between the adverse event or serious adverse event and the drugs. If a serious safety issue occurs during the trial, the sponsor and investigator must decide together whether to terminate the trial. The sponsor may also request to terminate the clinical trial according to the situation.

6.6 Serum Antibody Assays and Evaluations

This study will evaluate the potential immunogenicity of F-627 by testing serum anti-F-627 antibodies (IgG and IgM) in serum.

Serum antibody assays will be carried out at the laboratory of Generon (Shanghai) Corporation Ltd. Specific assay methods will be determined by the laboratory and will be described in detail in the clinical study report.

According to the study protocol, blood samples will be collected during screening (baseline value), and on days 8, 13, and 21 of each cycle. A total of 2.5 mL of blood will be collected.

Each time a total of 2.5 mL of blood is collected, transferred to a labeled serum separator tube, placed for about 30 minutes, and centrifuged at 1000 g for 15 minutes at room temperature. Aliquot the serum into two clean 1.5 mL EP tubes (approximately 0.3 mL of serum each), close the lid tightly and immediately transfer to ≤ –70 °C for storage. The entire sampling and serum collection process must be completed within 60 minutes. Samples must be transferred in a constant temperature box with dry ice. The collect tube label should include the following information: study number, subject initials, cycle number, day, sampling time (hour), etc.

7. ADVERSE EVENTS

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

7.1.1 Clinical adverse events

An AE 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 (investigational 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 CRFs. 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 guidelines to assess the severity of AEs that are not graded by NCI CTCAE:

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	The AE is life-threatening

7.1.1.2 Causality

The investigator will use "probably related", "possibly related", "unlikely related", and "unrelated" to assess the causality between adverse events and treatment. The evaluation criteria are shown in Appendix 1 (classification of causality between adverse events and drugs).

7.1.1.3 Serious adverse events

An AE meeting 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.

Death	An adverse event that causes the death (reporting of deaths resulting from progression of disease is exempted)
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, vomiting, diarrhea, influenza, or accidental injury (e.g. ankle sprains).
Important medical events requiring pharmaceutical or surgical interventions to prevent serious medical events	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 not requiring 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 drugs, interventions taken and outcome of an SAE should be included in the report.

7.1.2 Treatment and follow-up of adverse events

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

7.1.3 Abnormal values of laboratory measurements

7.1.3.1 Laboratory measurements

Results of laboratory measurement/vital signs should be recorded in the CRF. 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/vital signs should be recorded in CRFs as independent AEs if at least one of the followings are 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)
- Resulting in treatment interruption
- The investigator insists on reporting as an adverse event: If an abnormality in laboratory measurement/vital signs is associated with clinical symptoms/sign, the corresponding clinical symptom/sign should be reported as an adverse event, and the abnormal lab result or vital sign should be recorded in the case report form as supplemental information.

7.1.3.2 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 Adverse Event Management

7.2.1 Adverse event 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 investigational 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 investigational 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 adverse events and serious adverse events that occur from the signing of the informed consent form to 30 days after stopping the investigational drug should be collected, regardless of whether the event is observed by the investigator or self-reported by the subject.

7.2.2 Serious adverse event reporting (immediate)

If any clinical AEs or laboratory abnormalities that occur from the study to 30 days after the last dose are considered as SAEs, the investigator must report the SAEs to the sponsor and regulatory authorities in accordance with applicable regulations within 24 hours after learning of the event, regardless of whether interventions are given.

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

7.2.3 Special non-serious adverse event reporting

Progression of Disease

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

Lack of Treatment Efficacy

When the disease treated by the investigational drug worsens, it may not be possible to determine whether it is due to lack of treatment efficacy or an adverse event. In this case, it is generally considered to be due to lack of treatment efficacy rather than an adverse event unless the investigator believes that the exacerbation is related to the investigational drug.

Infusion Reaction

Other than reporting an "infusion reaction", symptoms and AEs associated with the investigational 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 CRFs;
- Severity of each AE;
- Symptoms that occur within 24 hours from the infusion, e.g. fever, chills, and hypotension.

Overdose

An overdose (whether accidental or intentional) must be reported according to procedures of treating the overdose, regardless of whether there are symptoms related to the overdose. All overdose-related symptoms should be reported as AEs.

7.2.4 Pregnancy

Subjects who become pregnant during the study must immediately notify the investigator. The investigational drug and chemotherapy drug must be discontinued. 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. STATISTICAL METHODS AND ANALYSIS

Descriptive statistics will be summarized for all variables obtained at various observation time points by dose cohorts, unless the protocol specifies that statistical analysis at a particular time point is not required. Overall, continuous variables (such as age) will be descriptively summarized with observed numbers, mean, median, standard deviation, minimum, and maximum. Categorical variables will be descriptively summarized with frequency and percentage based on each category. Continuous safety analysis of adverse events related to the investigational drug and other safety endpoints will be performed to determine how to achieve dose escalation.

The analysis dataset includes all enrolled subjects who have received at least one dose of the study drug. This dataset is available for all analyses. Statistical methods are detailed in the Statistical Analysis Plan.

8.1 Pharmacokinetic Evaluation

Two phases (single-dose and repeated dose); All PK parameters will be summarized using descriptive statistics by dose cohorts. The non-compartmental analysis of blood drug concentration data will be performed by the central laboratory using WinNonlin Enterprise.

For C_{max} (natural logarithm) of the single-dose and repeated-dose phase, $AUC_{(0\text{-}inf)}$, AUC_t, and AUC_{last} will be analyzed by one-way ANOVA, with dose cohort as the fixed factor. The least squares mean difference between the two dose cohorts and the 90% confidence intervals are obtained from the analysis of variance, and then the least squares geometric means ratio and the 90% confidence intervals are obtained by taking the antilog.

8.2 Pharmacodynamics Evaluation

Absolute neutrophil count (ANC) after administration in different dose cohorts, as well as the number of days of ANC less than 0.5×10^9/L, the number of days of ANC less than 1.0×10^9/L, and the time of ANC recovered to 1.0×10^9/L after the completion of chemotherapy in cycles 1 and 2–4 are observed.

8.3 Statistical Analysis of Safety Data

All adverse events are listed by patient, and coded using MedDRA as per physiologic system and standard terminology. Inferential statistics are not required for safety endpoints. Abnormal values laboratory measurements, vital signs, ECG, and other safety endpoints need to be noted.

9. DATA MANAGEMENT

This study will be monitored in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use - Good Clinical Practice (ICH-GCP). The clinical research associate will have direct access to source data and will verify the data by comparing data in case report forms with the data in the original medical records (patient's permitting the clinical research associate to directly access the data is part of the informed consent). This data verification process is an important part of ensuring the quality of the study. During this process, the investigator will be urged to correct any transcription errors and omissions. Also, the demographic data in case report forms are also considered as source data and should be verified before being transferred to the data management department.

9.1 Data Filling-In by Investigator

1) All parts of the case report forms should be carefully and detailedly filled in for subjects who signed the informed consent forms and are eligible to participate in the trial, with no blanks or omissions (blank spaces should be crossed out);
2) Data in the case report forms should be verified with medical records to ensure accuracy;
3) Case report forms are considered as source data. When making changes, the investigator should cross out incorrect data, write down the correct data, then sign and date the change;
4) Data that are significantly higher or beyond reference ranges should be verified and explained by the investigator;
5) Refer to the instructions for filling in case report forms (CRFs).

9.2 Requirement of Data Monitoring by Clinical Research Associate

1) The clinical research associate should check subjects' informed consent and screening procedures during the course of the trial;
2) Ensure that case report forms are filled out correctly and consistent with source data;
3) All errors or omissions have been corrected or noted, and signed and dated by the investigator;
4) Dose modifications, treatment changes, combined medications, and intercurrent diseases should be confirmed and documented for each subject;
5) Verify that withdrawal and loss to follow-up of enrolled subjects are explained in the case report forms;
6) Ensure that all adverse events have been documented, and serious adverse events have been documented and reported;
7) Verify whether the drugs are supplied, stored, dispensed, and returned in accordance with applicable regulations, and documented accordingly;
8) CRF must be completed for each enrolled patient;
9) CRF is filled out by the investigator and must be completed for each enrolled patient. After the completed CRF is reviewed by the clinical research associate, the original copy is handed over to the data management personnel of the statistical teaching and research section for data entry and data management, the middle copy is handed over to the sponsor for archiving, and the bottom copy is archived at the clinical pharmacological center.

9.3 Data Entry, Recording and Management

This study will use paper case report forms (pCRFs). Subject data will be clearly documented on the case report forms using blue or black ink. Correction fluid or tape must not be used. The clinical research associate of Generon (Shanghai) Corporation Ltd. will verify data during monitoring visits. The investigator should ensure that the data in the case report forms are accurate, complete, and clear. Data in the case report forms will be entered into the F-627 clinical study database and validated under the direction of data management personnel. Data in the case report forms that are missing, abnormal, or inconsistent will be presented to the investigator in data query forms and documented accordingly. The database is locked only after all queries have been resolved. The statistics department is responsible for data entry and management. The data manager will use software-compiled data entry programs for data entry and management. Double entry and proofreading should be performed by two data entry personnel independently to ensure the accuracy of data. The clinical research associate should submit any queries regarding case report forms to the investigator in data query forms. The investigator should respond and return the query as soon as possible. The medical statistician will then modify, confirm, and enter the data based on the investigator's response. The data query form can be issued again if necessary.

The revision and inquiry of the data query form should be documented. The data query form is an extension to the CRF and must be kept carefully.

9.4 Audits and Inspections

The authorized representative of Generon (Shanghai) Corporation Ltd., regulatory departments, independent ethics committee may audit or inspect the study site, including source data verification. The purpose of audits or inspections is to systematically and independently check all study-related procedures and documents to ensure that procedures have been implemented and data have been recorded, analyzed, and accurately reported in accordance with study protocol, GCP, ICH guidelines, and other laws and regulations. The investigator should immediately contact Generon (Shanghai) Corporation Ltd. when an inspection of the study site has been requested by the regulatory department.

9.5 Personnel Training

The principal investigator should maintain a study work record of study-related personnel (doctors, nurses, and other personnel). The principal investigator must ensure that all personnel have received appropriate training related to the study, and any new information related to the study has been communicated to the relevant personnel.

10. STUDY PROTOCOL REVISIONS

The study protocol and study procedures must not be modified without the consent of both the principal investigator and Generon (Shanghai) Corporation Ltd. If the study protocol must be modified, the amendments or new version of the protocol (revised protocol) must be reviewed and approved in writing by the ethics committee prior to being implemented. If applicable, it must be submitted to or approved by the local drug regulatory authorities according to local regulations.

If there is an administrative amendment, the change must be submitted to or approved in writing by the ethics committee as required. Generon (Shanghai) Corporation Ltd. and the ethics committee of the study site must be notified if amendments to the study protocol require changes to the study site's informed consent form. The application of the revised informed consent form must be approved by Generon (Shanghai) Corporation Ltd. and the ethics committee in writing.

Generon (Shanghai) Corporation Ltd. will distribute the protocol amendments and the new version of the study protocol to each principal investigator. The principal investigators will then be responsible for providing these documents to the ethics committee and other personnel at the study site.

11. STUDY MANAGEMENT

11.1 Clinical Study Agreement

The principal investigator of the study site must comply with all the terms and responsibilities of this clinical study agreement. The investigator must follow the study protocol when there are contradictions between the study protocol and the study agreement.

11.2 Study Schedule and Requirements for Early Termination

The study is scheduled to begin in December 2012 and end in October 2013. If the sponsor decides to terminate or suspend the study, the investigator and regulatory authorities must be notified in writing and the reason for early termination or suspension must be provided. The principal investigator/investigator must immediately explain the situation to subjects in this study, provide appropriate treatment, take necessary measures, and document the treatment measures in the source documents and case report forms.

12. ETHICS

12.1 Ethics of Clinical Study

The study must not be initiated before the protocol is approved by the Institutional Review Board (IRB) or ethics committee (EC). The composition of the IRB or EC must be in accordance with State Food and Drug Administration (SFDA) requirements and fulfill all duties required by the SFDA. The ethics committee approval should include comments on the review or approval, as well as the name, gender, and occupation of the committee members. The clinical research associate must first receive a copy of the ethics committee approval before distributing the drugs to the investigator. The investigator must receive the ethics committee approval before enrolling subjects.

According to applicable regulations of national regulatory authorities, in case of any amendments to the study protocol, the principal investigator is responsible for submitting to and obtaining written approval from the ethics committee for all study protocol amendments. The ethics committee must review advertisements for subject recruitment. The ethics committee must also re-approve the study protocol annually as required by local regulations. All SAEs or unexpected events that occur in the study must be reported to the EC as subject safety and the conduct of the trial may be affected. Consult the EC if the ethical aspects of the study need to be re-evaluated.

This study must be conducted in strict accordance with the requirements of SFDA "Good Clinical Practice" and the "Declaration of Helsinki".

12.2 Independent Ethics Committee (IEC)

Before the clinical study can begin, the trial protocol, informed consent form, and other materials provided to subjects must be reviewed and approved by the ethics committee. Relevant approvals must be provided to the sponsor.

12.3 Informed Consent Form (ICF)

The principal investigator of the study site must ensure that subjects receive adequate oral and written information regarding the nature, purpose, potential risks and benefits of the study, and must inform subjects that they may voluntarily withdraw from the study at any time. Subjects must be given the opportunity to ask questions and time to consider these information. The informed consent form must be signed and dated by each subject before the trial is initiated. The original copy must be kept by the investigator as part of the trial documents. A copy of the signature page is retained by the subject. The subject must be informed that the sponsor's designee may review the relevant medical records.

12.4 Privacy Protection

The informed consent form will state (or sometimes with separate documents) compliance with applicable data protection and privacy regulations. Based on this statement, subjects are required to authorize the investigator or other personnel who need to know the information to collect, use, and publish their data. The informed consent form will state that the study data are stored in a computer database and kept confidential in accordance with the applicable laws. In the database, subjects are only identified based on random number/study number/subject name initials. The informed consent form will also state that for the purpose of data verification, the sponsor's designee, regulatory authorities, and the ethics committee have direct access to hospital or medical records related to this study, including patient medical records.

13. QUALITY CONTROL OF CLINICAL TRIAL

In the study, the clinical research associate assigned by the sponsor will regularly conduct on-site monitoring visits to the study hospital to ensure that all parts of the study protocol are strictly implemented and the accuracy of study data.

The personnel participating in the study must receive uniform training, make unified records and adopt the same evaluation criteria. The investigator must fill out all parts of the CRF truthfully, detailedly, and carefully according to requirements to ensure that the contents of the case report form is authentic and reliable. The evaluation criteria for abnormalities in laboratory measurement should be based on the normal reference ranges provided by the measurer.

All observations and findings in the trial should be verified to ensure data reliability and to ensure that conclusions from the trial are derived from source data. Appropriate data management measures should be set in place during the clinical trial and data processing phases. Active measures should be taken to maintain a drop-out rate of < 20%.

14. Data Storage and Use

The investigator must agree to store all study data, including confirmation of all participating subjects (different records, such as CRFs and hospital source data, can be effectively verified), original informed consent forms of all patients, CRFs, and detailed drug dispensation records.

All data from this clinical trial are owned by Generon (Shanghai) Corporation Ltd. and must not be provided to persons unrelated to this trial in any form without the consent of the sponsor. Data from this study may be published only after the consent of the sponsor.

15. REFERENCES

1 National Comprehensive Cancer Network(NCCN), Version 2010.
2 L Roskos, B B Yang et al. Pharmacokinetic/Pharmacodynamic Modeling of Pegfilgrastim in Healthy Subjects. J. Clin Pharmacol 2006; 46: 747-757
3 F. A. Holmes, S. E. Jones, J. O'Shaughnessy et al. Comparable efficacy and safety profiles of once-per-cycle pegfilgrastim and daily injection filgrastim in chemotherapy-induced neutropenia: a multicenter dose-finding study in women with breast cancer. Ann Oncol. 2002 ;13:903-9.
4 Stephen E. Jones, Michael A. Savin et al. Phase III Trial Comparing Doxorubicin Plus Cyclophosphamide With Docetaxel Plus Cyclophosphamide As Adjuvant Therapy for Operable Breast Cancer. J Clin Oncol. 2006;24:5381-5387.
5 Martin M, Pienkowski P, Mackey J, et al. Adjuvant docetaxel for node-positive breast cancer. N Engl J Med 2005;352:2302-2313.
6 Nobuyuki Yamamoto, Ikuo Sekine, Kazuhiko Nakagawa, et al. A Pharmacokinetic and Dose Escalation Study of Pegfilgrastim (KRN 125) in Lung Cancer Patients with Chemotherapy-induced Neutropenia. Jpn J Clin Oncol 2009;39(7):425-430

Appendix

Appendix 1 Analysis of Causality Between Drugs and Adverse Events

1) Probably related:

This category applies to AEs that are considered highly likely to be caused by the drugs. 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 while drug-related toxicities persists 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 drugs, 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 meet 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", or "probably related".

	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	+	–	–	–

Appendix 2 ECOG Performance Status Scoring Standard

Score	Performance Status
0	Fully active, able to carry on all pre-disease 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; confined to bed for no more than 50% of waking hours
3	Capable of only limited self care; confined to bed or chair for more than 50% of waking hours
4	Completely disabled; cannot carry on any self care; totally confined to bed or chair
5	Death

Appendix 3 Summary of Protocol Amendments

Protocol Synopsis of Phase I Clinical Trial, Inclusion Criteria 4 (page 7)

Previous Text:

4) Absolute neutrophil count (ANC) ≥ 2.0×10^9/L, hemoglobin (Hb) ≥ 11.5 g/dL, and platelet (PLT) ≥ 100×10^9/L prior to chemotherapy.

Revised Text:

4) Absolute neutrophil count (ANC) ≥ 2.0×10^9/L, hemoglobin (Hb) ≥ 11.0 g/dL, and platelet (PLT) ≥ 100×10^9/L prior to chemotherapy.

Protocol Synopsis of Phase I Clinical Trial, Exclusion Criteria 11 (page 8) Previous Text:

11) History of tuberculosis (TB); history of TB exposure, unless negative for tuberculin test; TB patients undergoing treatment; or suspected TB evaluated by chest x-ray;

Revised Text:

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;

3.1 Overall Study Design and Schedule of Study Procedures, Figure 30-6 Schedule of F-627 study procedures (page 30)

Previous Text:

	1–15 Days Before Enrollment	Day 1, Day 22, Day 43, and Day 64 of the Study[6]	Day 3, Day 24, Day 45, and Day 66 of the Study[1]	Cycle 1 of Chemotherapy (Day 3–Day 21)[4]	Cycles 2–4 of Chemotherapy (Day 3–Day 21)[5]	End of Study (Day 84)[7]
Signing Informed Consent Form	X					
History of Cancer	X					
Complete Physical Examination	X					X
Physical Examination		X	X			
Abdominal Ultrasound[2]	X	X				X
Chemotherapy		X				
Urinalysis[2]	X	X				X
Administration[1]			X			
12-Lead ECG[3]	X					X
Color Doppler Echocardiography	X					
Chest X-Ray	X					X
Height and Weight[2]	X	X				

	1–15 Days Before Enrollment	Day 1, Day 22, Day 43, and Day 64 of the Study[6]	Day 3, Day 24, Day 45, and Day 66 of the Study[1]	Cycle 1 of Chemotherapy (Day 3–Day 21)[4]	Cycles 2–4 of Chemotherapy (Day 3–Day 21)[5]	End of Study (Day 84)[7]
Body Temperature	X	X	X	X	X	X
Routine Blood Test (including ANC)	X	X	X	X	X	X
Clinical Chemistry[2]	X	X				X
Serum Pregnancy Test	X					X
Blood Pressure and Heart Rate	X	X				X
Pharmacokinetics[8]			X	X	X	
Serum Antibody[9]		X	X	X	X	X
AEs and Combined Medications[9]		X	X	X	X	X

1. Three dose cohorts for F-627 (80 μg/kg, 240 μg/kg and 320 μg/kg);
2. Examination should be completed upon enrollment and the start of each chemotherapy cycle. Body height is measured upon enrollment only, while body weight is measured on day 1 of each cycle; for abdominal ultrasound, a retest on day 1 of cycle 1 is not required and the baseline result can be used; for routine blood test and clinical chemistry before the start of cycle 1, results within 7 days are acceptable;
3. 12-lead ECG should be repeated at the last visit of the study;
4. For chemotherapy cycle 1, starting from day 3, oral temperature measurement and routine blood test will be performed daily until ANC recovers to > 1.0×10^9/L from nadir, and once every 3 days thereafter until the next cycle;
5. For chemotherapy cycles 2–4 (day 3–day 21 of each chemotherapy cycle, i.e., day 24–day 84 of the study), starting on each day 3 of cycles 2–4, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to > 1.0×10^9/L, and once every 3 days thereafter until the next cycle;
6. Only when ANC of a subject recovers to > 1.0×10^9/L as judged by the investigator, can the next cycle start;
7. The last visit is on day 84, and the subjects should complete a follow-up visit by telephone on day 114 (30 days after the last visit);
8. In cycle 1 and cycle 3, blood samples are collected for PK at time points shown in Table 30-8;
9. Blood samples will be collected for serum antibody assays during screening or before treatment with the drugs, as well as on days 8, 13, and 21 of each cycle.

Remark: All laboratory measurements will be performed at a central laboratory assigned by the investigator. In case of any toxicities related to chemotherapy, subsequent treatment can be modified according to common diagnosis and treatment practice in the judgment of the investigator.

Revised Text:

	1–15 Days Before Enrollment	Day 1, Day 22, Day 43, and Day 64 of the Study (Window Phase: 3 Days Before Dosing)[6]	Day 3, Day 24, Day 45, and Day 66 of the Study[1]	Cycle 1 of Chemotherapy (Day 3–Day 21)[4]	Cycles 2–4 of Chemotherapy (Day 3–Day 21)[5]	End of Study (Day 84)[7]
Signing Informed Consent Form	X					
History of Cancer	X					
Complete Physical Examination	X					X
Physical Examination		X	X			

	1–15 Days Before Enrollment	Day 1, Day 22, Day 43, and Day 64 of the Study (Window Phase: 3 Days Before Dosing)[6]	Day 3, Day 24, Day 45, and Day 66 of the Study[1]	Cycle 1 of Chemotherapy (Day 3–Day 21)[4]	Cycles 2–4 of Chemotherapy (Day 3–Day 21)[5]	End of Study (Day 84)[7]
Abdominal Ultrasound[2]	X	X				X
Chemotherapy		X				
Urinalysis[2]	X	X				X
Administration[1]			X			
12-Lead ECG[3]	X					X
Color Doppler Echocardiography	X					
Chest X-Ray	X					X
Height and Weight[2]	X	X				
Body Temperature	X	X	X	X	X	X
Routine Blood Test (including ANC)	X	X	X	X	X	X
Clinical Chemistry[2]	X	X				X
Serum Pregnancy Test	X					X
Blood Pressure and Heart Rate	X	X				X
Pharmacokinetics[8]			X	X	X	
Serum Antibody[9]		X	X	X	X	X
AEs and Combined Medications[9]		X	X	X	X	X

1. Three dose cohorts for F-627 (80 μg/kg, 240 μg/kg and 320 μg/kg);
2. Examination should be completed upon enrollment and the start of each chemotherapy cycle. Body height is measured upon enrollment only, while body weight is measured on day 1 of each cycle; for abdominal ultrasound, a retest on day 1 of cycle 1 is not required and the baseline result can be used; for routine blood test and clinical chemistry before the start of cycle 1, results within 7 days are acceptable;
3. 12-lead ECG should be repeated at the last visit of the study;
4. For chemotherapy cycle 1, starting from day 3, oral temperature measurement and routine blood test will be performed daily until ANC recovers to > 1.0×10^9/L from nadir, and once every 3 days thereafter until the next cycle;
5. For chemotherapy cycles 2–4 (days 3–21 of each chemotherapy cycle, i.e., days 24–84 of the study), starting from each day 3 of cycles 2–4, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to > 1.0×10^9/L, and once every 3 days thereafter until the next cycle;
6. The investigator may decide to start next cycle only when ANC recovers to > 1.0×10^9/L;
7. The last visit is on day 84, and the subjects should complete a follow-up visit by telephone on day 114 (30 days after the last visit);
8. In cycle 1 and cycle 3, blood samples are collected for PK at time points shown in Table 30-8;
9. Blood samples will be collected for serum antibody assays on days 1, 8, and 13 of each cycle as well as day 22 of chemotherapy cycle 4.

Remark: All laboratory measurements will be performed at a central laboratory assigned by the investigator. In case of any toxicities related to chemotherapy, subsequent treatment can be modified according to common diagnosis and treatment practice in the judgment of the investigator.

4.1 Study Population and Number of Subjects (page 30)

Previous Text:

The investigator must ensure that the first cycle of treatment follows the recommended chemotherapy regimen; in chemotherapy cycles 2–4, treatment regimen and dose are permitted to be individualized based on subject conditions. Dose delay and one dose reduction due to toxicities other than myelotoxicity (such as cardiotoxicity) are permitted in this trial. If the subject requires a second dose reduction, the investigator and sponsor must decide together whether the subject should continue the treatment with the drugs.

Revised Text:

The investigator must ensure that the first cycle of treatment follows the recommended chemotherapy regimen; in chemotherapy cycles 2–4, treatment regimen and dose are permitted to be individualized based on subject conditions. Dose delay and one dose reduction due to chemotherapy-induced toxicities (such as cardiotoxicity) are permitted in this trial. If the subject requires a second dose reduction, the investigator and sponsor must decide together whether the subject should continue the treatment with the drugs.

4.2 Inclusion Criteria 4 (page 30)

Previous Text:

4) Absolute neutrophil count (ANC) $\geq 2.0 \times 10^9$/L, hemoglobin (Hb) $\geq$ 11.5 g/dL, and platelet (PLT) $\geq 100 \times 10^9$/L prior to chemotherapy.

Revised Text:

4) Absolute neutrophil count (ANC) $\geq 2.0 \times 10^9$/L, hemoglobin (Hb) $\geq$ 11.0 g/dL, and platelet (PLT) $\geq 100 \times 10^9$/L prior to chemotherapy.

4.3 Exclusion Criteria 11 (page 31)

Previous Text:

11) History of tuberculosis (TB); history of TB exposure, unless negative for tuberculin test; TB patients undergoing treatment; or suspected TB evaluated by chest x-ray;

Revised Text:

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;

5.5 Drug Preparation (pages 32–33)

Previous Text:

The pharmacist is responsible for preparing the drugs and the nurse is responsible for reviewing. During drug preparation, inject 0.5 mL of water for injection slowly into the vial along the wall, let the vial stand for 1–2 minutes, and perform subcutaneous injection after the powder is completely dissolved. The injection should be used within 1 hour after preparation. The recommended concentration for F-627 is 2.0 mg/mL. The drug is generally injected in the morning (08:00 ± 1:00). The subject should remain in a sitting or walking position within two hours after the injection.

Revised Text:

The pharmacist is responsible for preparing the drugs and the nurse is responsible for reviewing. During drug preparation, inject 0.5 mL of water for injection slowly into the vial along the wall, let the vial stand for 1–2 minutes, and perform subcutaneous injection after the powder is completely dissolved. The injection should be used within 1 hour after preparation. See the drug preparation procedure manual. The drug is generally injected in the morning (09:00 ± 1:00). The subject should remain in a sitting or walking position within two hours after the injection.

6.3 Clinical Evaluationss During and After F-627 Treatment (page 35)

Previous Text:

For chemotherapy cycles 2–4 (days 3–21 of each chemotherapy cycle, i.e., days 23–84 of the study), starting from day 2, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to > 1.0×10^9/L, and once every 3 days thereafter until the next cycle. Evaluations include:

Revised Text:

For chemotherapy cycles 2–4 (days 3–21 of each chemotherapy cycle, i.e., days 23–84 of the study), starting from day 3, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to > 1.0×10^9/L, and once every 3 days thereafter until the next cycle. Evaluations include:

6.3.3 Blood sampling of single-dose and repeated-dose PK studies, Table 30-8 (page 38)

Previous Text:

Study Process	Days of Study	Days of Dosing	Blood Sampling Time Point of Cycle 1 (hours: minutes)	Blood Sample Number	Blood Sampling Time Point of Cycle 3 (hours: minutes)	Blood Sample Number
				F-627 PK Study (2.5 mL)		F-627 PK Study (2.5 mL)
Study Process	**3**	1	–01:00±0.1:00	PK 1	00:00±0.1:00	PK 1
			02:00±0.3:00	PK 2	02:00±0.3:00	PK 2
			06:00±0.5:00	PK 3	06:00±0.5:00	PK 3
			12:00±0.5:00	PK 4	12.00±0.5:00	PK 4
	4	2	24:00±0.5:00	PK 5	24:00±0.5:00	PK 5
			36:00±0.5:00	PK 6	36:00±0.5:00	PK 6
	5	3	48:00±1:00	PK 7	48:00±1:00	PK 7
	6	4	72:00±1:00	PK 8	72:00±1:00	PK 8
	7	5	96:00±1:00	PK 9	96:00±1:00	PK 9
	8	6	120:00±1:00	PK 10	120:00±1:00	PK 10
	9	7	144:00±1:00	PK 11	144:00±1:00	PK 11
	11	9	192:00±2:00	PK 12	192:00±2:00	PK 12
	13	11	240:00±2:00	PK 13	240:00±2:00	PK 13
Number of Blood Samples Collected on Each Subject				13		13
Total Volume of Blood Samples Collected on Each Subject				32.5mL		32.5 mL

Revised Text:

Study Process	Days of Study	Days of Dosing	Blood Sampling Time Point of Cycle 1 (hours: minutes)	Blood Sample Number F-627 PK Study (2.5 mL)	Blood Sampling Time Point of Cycle 3 (hours: minutes)	Blood Sample Number F-627 PK Study (2.5 mL)
Study Process	**3**	1	–01:00±0.1:00	PK 1	00:00±0.1:00	PK 1
			02:00±0.3:00	PK 2	02:00±0.3:00	PK 2
			06:00±0.5:00	PK 3	06:00±0.5:00	PK 3
			12:00±0.5:00	PK 4	12.00±0.5:00	PK 4
	4	2	24:00±0.5:00	PK 5	24:00±0.5:00	PK 5
			36:00±0.5:00	PK 6	36:00±0.5:00	PK 6
	5	3	48:00±1:00	PK 7	48:00±1:00	PK 7
	6	4	72:00±1:00	PK 8	72:00±1:00	PK 8
	7	5	96:00±1:00	PK 9	96:00±1:00	PK 9
	8	6	120:00±1:00	PK 10	120:00±1:00	PK 10
	9	7	144:00±1:00	PK 11	144:00±1:00	PK 11
	11	9	192:00±2:00	PK 12	192:00±2:00	PK 12
	13	11	240:00±2:00	PK 13	240:00±2:00	PK 13
	16	14	312:00±2 4:00	PK 14	312:00±24:00	PK 14
	21	19	432:00±2 4:00	PK 15	432:00±24:00	PK 15
Number of Blood Samples Collected on Each Subject				15		15
Total Volume of Blood Samples Collected on Each Subject				37.5 mL		37.5 mL

Title Page

Clinical Study Protocol

A Single-Center, Open-Label, Dose-Escalation Phase I Clinical Trial of Recombinant Human Granulocyte Colony Stimulating Factor-Fc Fusion Protein for Injection as an Adjuvant to Chemotherapy in Subjects with Breast Cancer

Protocol Number:	**2012-F-627-CH1**	
Investigational Drug:	Recombinant human granulocyte colony stimulating factor-Fc fusion protein (F-627)	
Clinical Phase:	Phase I	
Current Version No.:	2.5	
Issue Date of Current Version:	Dec. 3, 2012	
Original Version No./ Issue Date:	0.0/Apr. 25, 2010	
Study Site:	Fudan University Shanghai Cancer Center Address: No. 270 Dong'an Road, Shanghai, China	Postal Code: 200032
Principal Investigator	Professor Cao Junning	Tel.: +86-021-61733900
Sponsor	Generon (Shanghai) Corporation Ltd. Address: Building 9, No. 787 Kangqiao Road, Shanghai, China	Postal Code: 201315
Contact	Huang Cheng	Tel.: +86-021-61760866

Signature Page

Protocol Title: A Single-Center, Open-Label, Dose-Escalation Phase I Clinical Trial of Recombinant Human Granulocyte Colony Stimulating Factor-Fc Fusion Protein for Injection as an Adjuvant to Chemotherapy in Subjects with Breast Cancer

Protocol Number: 2012-F-627-CH1

I confirm that I have read, understood and agreed to abide by all provisions in the Clinical Study Protocol (Number: 2012-F-627-CH1, Date: Dec. 3, 2012). I shall carefully carry out my duties in accordance with the Good Clinical Practice.

Principal Investigator

Cao Junning _____ 2012.12.4
Signature Date

Cao Junning
Principal Investigator (Printed Name)

Sponsor

Huang Cheng _____ 2012.12.4
Signature Date

Huang Cheng

Emergency Contacts of Generon (Shanghai) Corporation Ltd.

In case of a medical emergency, the clinical study leading official may be contacted. If the director is not available, the protocol writer or the clinical research associate (CRA) may be contacted via the information shown in the table below.

	Name	Contact Number
Project leading official	Huang Cheng	021-6176-0866 or 18621697558
Protocol Writer	Tang Kaiyang/ Huang Cheng	021-6176-0866 or 18621697558
24-Hour Emergency Contact Number	Huang Cheng	021-6176-0866 or 18621697558

Synopsis of Phase I Clinical Study Protocol

Investigational Drug	Recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection
Protocol No.	**2012-F-627-CH1**
Title	A single-center, open-label, dose-escalation phase I clinical trial of recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection as an adjuvant to chemotherapy in subjects with breast cancer
Mechanism of Drug Action	The recombinant human granulocyte colony stimulating factor (rhG-CSF)-Fc fusion protein is a long-acting rhG-CSF expressed in CHO cells. Each molecule of rhG-CSF-Fc fusion protein contains two G-CSF dimer molecules, which may overcome the weak bioactivity of pegfilgrastim and produce a stronger receptor activation signal, thereby accelerating the differentiation and proliferation of neutrophils in bone marrow. Also, preclinical studies have shown that the pharmacokinetic and pharmacodynamic properties of rhG-CSF-Fc is different from or superior to pegfilgrastim, and therefore it decreases the severity of neutropenia and reduces the duration of severe neutropenia in cancer patients after chemotherapy.
Study Site	Fudan University Shanghai Cancer Center
Pharmacokinetic Analysis Site	Covance Pharmaceutical Research and Development (Shanghai) Co., Ltd.
Number of Planned Enrollment	It is planned to enroll 18 subjects
Study Population	Female postoperative patients with breast cancer who receive adjuvant chemotherapy will be enrolled in this study. Eligible patients should have received no chemotherapy or only one chemotherapy. The chemotherapy used for this trial is EC→P or EC→T, 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 all 4 cycles, subjects will receive 4 subsequent cycles of sequential chemotherapy (either paclitaxel or docetaxel) and supportive care according to routine practice.
Objectives	**Primary Objective:** To evaluate the safety and tolerability of recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection (F-627) in treatment of female postoperative breast cancer patients who require adjuvant chemotherapy in a single-center, open-label, single-dose and repeated-dose, dose-escalation phase I clinical trial.
	Secondary Objectives: 1. To evaluate the pharmacokinetics of F-627 by determining the serum drug concentrations of F-627 at different time points after dosing; 2. To evaluate the pharmacodynamics of F-627 by analyzing the relationship between serum drug concentrations of F-627 and neutrophil counts in blood at different time points after dosing, and to provide a recommended dose for phase II clinical trial.
	Exploratory Objectives: 1. To observe the number of days in which absolute neutrophil count is less than 0.5×10^9/L in cycle 1 of chemotherapy, the average time of ANC recovery, and the number of days in which absolute neutrophil count is less than 1×10^9/L, and to provide guiding significance for phase II clinical trial. 2. To evaluate the potential immunogenicity of F-627 by testing anti-F-627 antibodies (IgG and IgM) in serum.

Dosage and Administration	This study includes 3 dose cohorts, 80, 240, and 320 μg/kg, each of which will enroll 6 patients with breast cancer sequentially receiving adjuvant chemotherapy. Subjects in each cohort will receive a single-dose of F-627 by subcutaneous injection approximately 48 hours after the completion of chemotherapy. Blood samples are then collected at multiple time points during the subsequent follow-up visits, to evaluate the pharmacokinetics, pharmacodynamics, and safety of the drug. If dose-limiting toxicities are not observed before the start of cycle 2, then the same dose of F-627 is to be given at approximately 48 hours after each chemotherapy in cycles 2-4.
Dose-Escalation	The starting dose is 80 μg/kg. Only after 6 subjects have completed the treatment and observation of the first cycle, the sponsor and investigator will determine whether to proceed to the next higher dose based on the safety evaluation. This is also applicable to the 240 μg/kg cohort. Dose escalation is not pursued after 6 subjects in the 320 μg/kg cohort complete the evaluation. Dose escalation should be stopped if 2 cases of DLTs are observed in the first cycle in each cohort.
Rationale for Dose Selection	Results from 3-month repeat dosing toxicity studies showed that the no-observed-adverse-effect-levels (NOAEL) of F-627 was 1000 μg/kg in rats and 675 μg/kg in cynomolgus monkeys. F-627 is a recombinant fusion protein with a molecular weight of 95000 Daltons. Results from preclinical studies showed that the toxicity of F-627 was due to the amplification of pharmacodynamics, which is closely associated with the dose (expressed in mg/kg) in various different species. The maximum safe starting dose of F-627 was calculated to be 67.5 μg/kg when converted using mg/kg, which is one-tenth of the NOAEL in cynomolgus monkeys. In a phase I clinical trial in healthy volunteers, male subjects received a single-dose of F-627 by subcutaneous injection at doses of 30, 60, 120, 240, and 360 μg/kg. Results showed that neutrophil increased across all dose cohort up to 96 hours following the administration of F-627. The maximum dose of 360 μg/kg was safe and no serious adverse events observed. Higher incidence rate of adverse event was observed with the increasing dose. Low dose was better tolerated than high dose. According to the animal studies and phase I clinical trial, 80 μg/kg is safe as the starting dose for patients. Firstly, the maximum dose should not exceed the maximum dose determined in the phase I clinical trial. Secondly, the G-CSF mole number of 240 μg/kg F-627 is comparable to that of 100 μg/kg pegfilgrastim. Finally, 60 μg/kg cohort exhibited superior pharmacodynamics than the 150 μg/kg cohort in treating the chemotherapy-induced neutropenia in monkey models.
Dose-Limiting Toxicity	Dose-limiting toxicity (DLT) refers to an intolerable toxicity that is experienced during the treatment with investigational drug and limits the further dose escalations. DLT is defined as any grade 3 or greater adverse event related to the investigational drug that observed in cycle 1 (21 days). Adverse events will be assessed according to NCI CTCAE V4.03 criteria. After 6 subjects in each dose of F-627 cohort have completed the treatment and observation of first cycle, the trial may proceed into the next dose cohort only if less than 2 subjects develop DLT. If a grade 3 or greater adverse event related to the investigational drug is observed in cycles 2-4, then the investigator and sponsor will determine together whether the adverse event would affect further dose escalations.
Safety Evaluation	Safety endpoints include laboratory measurements, physical examinations, vital signs, and performance status, as well as adverse events. The incidence rate and severity of adverse events (AEs) will be assessed according to NCI CTCAE 4.03 criteria.
Salvage Therapy	During the study, an approved G-CSF treatment should be given to the patient as salvage therapy in the case of febrile neutropenia (defined as ANC $< 1.0 \times 10^9$/L; a single measurement of body temperature > 38.3 °C or a fever ≥ 38.0 °C sustained for longer than 1 h; Note: temperature measurements is based on oral temperature or equivalent armpit/rectal temperature) or grade 4 neutropenia lasting greater than 3 days. The sponsor recommends GRAN® (filgrastim) at a dose equivalent to 5 μg/kg/day of G-CSF, once daily for ≤ 2 weeks or until neutrophil count recovers to 1.0×10^9/L.

Inclusion Criteria	All of the following conditions must be met: 1) 18-75 years old; 2) Female postoperative breast cancer patients who require adjuvant chemotherapy, and are planned to receive 4 cycles of EC chemotherapy; 3) ECOG performance status of 0-1; 4) Absolute neutrophil count (ANC) $\geq 2.0 \times 10^9$/L, hemoglobin (Hb) $\geq$ 11.5 g/dL, and platelet (PLT) $\geq 100 \times 10^9$/L prior to chemotherapy 5) Hepatic and renal function within the normal range; 6) Left ventricular ejection fraction greater than 50%; 7) Willing to sign the informed consent form and able to comply with protocol requirements;
Exclusion Criteria	Subjects who meet any of the following must be excluded from this study: 1) Women in pregnancy or breastfeeding; Women of child–bearing potential have a positive pregnancy test result prior to the first dose; 2) Life expectancy less than 12 months; 3) Radiation therapy within 4 weeks prior to enrollment; 4) Breast cancer patients who have received neoadjuvant chemotherapy before radical mastectomy; 5) Prior bone marrow or stem cell transplant; 6) With other malignant tumors other than breast cancer; 7) Have received G-CSF treatment within 6 weeks prior to enrollment; 8) Diagnosed with acute congestive heart failure, cardiomyopathy, or myocardial infarction by clinical diagnosis, ECG or other approaches; 9) With any disease that may cause splenomegaly; 10) With acute infection, chronic active Hepatitis B within 1 year (unless patients tested negative for HBsAg prior to enrollment), or Hepatitis C; 11) History of tuberculosis (TB); history of TB exposure, unless negative for tuberculin test; TB patients undergoing treatment; or suspected TB evaluated by chest x-ray; 12) Known HIV positive or AIDS; 13) With sickle cell anemia; 14) With alcohol or drug abuse that may affect the compliance with the study; 15) With known hypersensitivity to E. coli derived proteins, G-CSF, or excipients; 16) Has received any other investigational drug within 4 weeks prior to enrollment; 17) Patients with diseases or symptoms unsuitable for participating in the clinical trial based on the investigator's judgment;
Criteria for Terminating Study	1) Incidence rate and severity of serious adverse events (SAEs) indicate the study should be terminated early as determined by the investigators and the sponsor; 2) The dose-limiting toxicity of the subjects does not recover or cannot be relieved; 3) The investigators question the safety of the drug and believe that the continuation of the study may pose serious risks to the subjects; 4) The maximum dose set in the clinical trial has been reached; 5) Data fraud, or inaccurate/incomplete collection of data;
Criteria for Subject Withdrawal	Subjects will withdraw from the study with a written explanation when the following conditions occur. 1) Subject withdraws voluntarily during the trial; 2) The investigator considers that withdrawal is for the best interest of the subject; 3) The investigator or subject believes that to continue the trial may result in intolerable adverse events; 4) Complications or worsening co-morbidities affecting subject's participation occur; 5) Subject is found to violate protocol after enrollment, or a major protocol violation occurs during the trial; 6) When a safety concern regarding the investigational drug arises, but data is yet unknown, resulting in potential risks if subjects continue the trial;

Drop-Out Criteria	Determination of drop-outs: Eligible subjects who have signed the informed consent form and been enrolled have the right to withdraw from the study at any time. Subjects who do not complete the entire observation are considered drop-outs regardless of the time or reason of the withdrawal. A replacement should be implemented immediately according to the original plan when a drop-out occurs. Blood samples from drop-out subjects should be retained.
Evaluations:	
Safety Evaluation	Medical consultation, physical examinations, vital signs, laboratory measurements (hematology, clinical chemistry, routine urinalysis, etc.), weight, ECG, abdominal ultrasound and adverse event evaluation.
Pharmacokinetic Evaluation	Serum: C_{max}, T_{max}, MRT, V_d, Kel, Tl/2z, AUC_{last}, AUC, CL/F, Vz/F
Pharmacodynamic Evaluation	Absolute neutrophil count (ANC) after dosing, as well as the number of days in which ANC is less than 0.5×10^9/L, the number of days in which ANC is less than 1.0×10^9/L, and the time ANC recovers to 1.0×10^9/L after chemotherapy in cycles 1 and 2-4 are observed.
Statistical Analysis: Descriptive statistics will be summarized for all variables obtained at various observation time points by dose cohorts, unless the protocol specifies that statistical analysis at a particular time point is not required. Overall, continuous variables (such as age) will be descriptively summarized with observed numbers, mean, median, standard deviation, minimum, and maximum. Categorical variables will be descriptively summarized with frequency and percentage based on each category. Continuous safety analysis of adverse events related to the investigational drug and other safety endpoints will be performed. The analysis dataset includes all enrolled subjects who have received at least one dose of the study drug. This dataset is available for all analyses. Statistical methods are detailed in the Statistical Analysis Plan.	
Pharmacokinetics	Two phases (single-dose and repeated dose); All PK parameters will be summarized using descriptive statistics by dose cohorts. The non-compartmental analysis of blood concentration data will be performed by the central laboratory using WinNonlin Enterprise. C_{max} (natural logarithm), $AUC_{(0\text{-inf})}$, AUC_t, and AUC_{last} of the single-dose and repeated-dose phase were analyzed using one-way ANOVA, with dose cohort as the fixed factor. The least squares mean difference between the two dose cohorts and the 90% confidence intervals are obtained from the analysis of variance, then the least squares geometric means ratio and the 90% confidence intervals are obtained by taking the antilog.
Pharmacodynamic Evaluation	The number of days in which ANC is less than 0.5×10^9/L, the number of days in which ANC is less than 1×10^9/L, and the mean time of ANC recovery to 1.0×10^9/L in chemotherapy of cycles 1 and 2-4 of chemotherapy are calculated for each dose cohort. ANC profiles in various dose cohorts are plotted.
Safety Evaluation	All adverse events are listed by patient, and coded by MedDRA as per System Organ Class and Preferred Term. Inferential statistics are not required for safety endpoints. Abnormal values of laboratory measurements, vital signs, ECG, and other safety endpoints need to be noted. The incidence rate and severity of adverse events (AEs) will be assessed according to NCI CTCAE 4.03 criteria.

Table of Contents

List of Tables

List of Figures

Abbreviations and Terms

Abbreviations and Terms	Definitions
AEs	Adverse Events
ALP	Alkaline Phosphatase
ANC	Absolute Neutrophil Count
AUC	Area Under Concentration-Time Curve
CL/F	Total Clearance
C_{max}	Maximum Serum Concentration
DHL	Lactate Dehydrogenase
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ED_{50}	Median Effective Dose
EMEA	European Medicines Agency
FDA	U.S. Food & Drug Administration
GCP	Good Clinical Practice
Kel	Elimination rate constant
MRT	Mean Residence Time
NCI CTC	National Cancer Institute Common Terminology Criteria
NOAEL	No-Observed-Adverse-Effect Level
PK/PD	Pharmacokinetics/Pharmacodynamics
Pubmed	PubMed search system developed by the National Institutes of Health
rhG-CSF-Fc	Recombinant human granulocyte colony stimulating factor-Fc fusion protein
SFDA	State Food and Drug Administration
T_{max}	Time to Peak
V_d	Apparent Volume of Distribution
V_z/F	Volume of Distribution
WBC	White Blood Cell
WHO	World Health Organization

1. STUDY BACKGROUND

1.1 Introduction to F-627

1.1.1 Name, structure, and physicochemical properties

All recombinant human granulocyte colony stimulating factors (rhG-CSF) that have already been approved for marketing in China and other countries, and the more than 10 rhG-CSFs in clinical and pre-clinical studies, are monomeric G-CSFs (Pharmaproject ref). This rhG-CSF-Fc fusion protein, is a rhG-CSF dimer expressed in CHO cells, based on the technology of Fc fusion, whose IP is protected by Chinese patents. The Fc fragment is derived from human immunoglobulin with prolonged effects. The half-life of IgG immunoglobulins may be up to 3 weeks in human blood. Recombinant protein drugs generated using the Fc fusion protein technology such as Enbrel (for rheumatoid arthritis, soluble TNFα receptor and IgG_1) and chimeric monoclonal antibodies such as Remicade (for rheumatoid arthritis, anti-TNFα IgG_1) and Abciximab (for platelet aggregation, anti-GPIIb/IIIa IgG_1) have been successfully marketed. In addition, D-Mab (for osteoporosis, anti-RANKL IgG_2) has been approved for marketing by FDA. The safety and efficacy of Fc fusion protein have been validated for clinical application.

The Fc region of human immunoglobulin mediates effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and plays an important role in the immune defense. ADCC refers to the phagocytosis or killing of target cells, which is mediated by the specific binding of antibody or the Fc fusion protein via the Fc region to the Fcγ receptor expressed on the surface of effector cells, such as natural killer cells and macrophages. CDC refers to the killing of target cells by the activation of complements, which is mediated by the specific binding of antibody or the Fc fusion protein via the Fc region to plasma proteins, such as complement proteins C1q, C3 and C4.

The effector function of the Fc fragment in rhG-CSF-Fc is reduced and eliminated by two methods (U.S. Patent: 6797493, Chinese Patent: 02126839.8). Among the four human IgGs, IgG2 has a weaker effector function. In regard to FcγRI and FcγRIIB, the affinity of FcγRI for the four human IgGs is in the order of: IgG1, IgG3 > IgG4 >> IgG2. The affinity of FcγRIIB for the four human IgGs is: IgG3 > IgG1 > IgG4 > IgG2 (Van Sorge et al 2003, Tissue Antigens. Vol. 61:189–202). Therefore, IgG2 Fc was used for the making of the fusion protein. Given the substitution of proline (a proven functional residue in CDC) at position 331 of IgG2 with serine, the fusion protein generated by this method no longer has any effector function. The above amino acid number (e.g. position 331) was calculated using the EU numbering system described by Kabat et al. (Sequence of Proteins of Immunological Interest, edition5, United States Department of Health and Human Services, 1991).

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. The sequence of this linker is: GlySerGlyGlyGlySerGlyGlyGlyGlySerGlyGlyGlyGlySer. The purpose is 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 also allow one G-CSF to be distant from another G-CSF, and produce rhG-CSF in a dimeric form with two G-CSF molecules on a single Fc fragment. The rhG-CSF-Fc dimer may cause a stronger receptor activation compared with a 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, 812-amino acid single-chain receptor with high affinity to G-CSF. During neutrophil development, each cell expresses about 50–500 G-CSFR. The more mature neutrophils are, the higher the density of G-CSFR on their surfaces Tamada et al. performed a 2.8A^{o} diffraction analysis of the G-CSF: G-CSFR crystal (PNAS, 2008, Vol. 103: 3135-3140) and found that this complex exists in a 2:2 ratio, i.e., 2 ligands with 2 receptors (Fig. 3-2). Each G-CSF molecule binds to one receptor. Only when two receptors binding to the 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). JAK2 then initiates gene transcription by activating STAT3, resulting in cell proliferation. Theoretically, dimeric ligands may be able to 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.

Furthermore, in a clinical study of rhG-CSF (Schwinger et al, 1993, Bone Marrow Transplantation, Vol. 11:489-492), it was found that peripheral blood precursor cells and peripheral blood stem cells increase drastically after the injection of rhG-CSF, which is called 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 the character of bone marrow matrix proteins and adhesion molecules have the similar function of PBPC and/or PBSC mobilization. Therefore, peripheral blood cells collected after rhG-CSF injection, are enriched with PBPCs and PBSCs, and are widely used as donor cells for conventional bone marrow transplantation in developed countries.

1.1.3 Preclinical pharmacokinetics study

Pharmacokinetics 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. Blood drug concentration and ANC increase (PD response) is dose-dependent. In the study of F-627 (100 μg/kg) subcutaneous injection in rats, $T_{1/2}$ was 7.6 ± 1.3 hours, C_{max} was 162 ng/mL, and AUC was 4217 ± 641 ng/mL·*hr. In the study of pegfilgrastim (Neulasta, 100 μg/kg) in rats, $T_{1/2}$ was 7.1 hours (Zamboni W.C, 2003, Pharmacotherapy, Vol.23 (8 pt2):9S-14S) and AUC was 1600 ng/mL·*hr (FDA IND). In cynomolgus monkey models with cyclophosphamide-induced neutropenia, the PK parameters of F-627 and pegfilgrastim at the equivalent dose were comparable (as shown in Table 30-1). However, F-627 resulted in a faster neutrophil recovery compared with pegfilgrastim.

Table 30-1. Comparison of PK parameters between F-627 and pegfilgrastim.

Dose 60 μg/kg	T_{max} (hr)	C_{max} (ng/mL)	AUC (ng/mL·hr)	$T_{½}$ (hr)
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 toxicology study

A systematic preclinical safety evaluation of F-627 has been conducted according to SFDA's registration categories of biological products and requirements for submission dossiers. Refer to Table 30-2 for the details of safety evaluation tests. In the repeat dosing toxicity studies in rats and cynomolgus monkeys, application requirements of FDA were taken into account by increasing the number of animals and the number of organs dissected. Meanwhile, the validation of PK/TK analysis method was in accordance with the application requirements of FDA/EMEA. 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.

Table 30-2. 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	Repeat dosing toxicity	75,225,675	Repeat	Administration:	675

Animal	Study Title	Dose (μg/kg)	Administration	Observation Time	NOAEL μg/kg
monkey	study			3 months Recovery period: 1 month	
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 for 5 times once every other day	30 minutes, 3 hours	
Rabbit	RBC in vitro hemolysis test	1000 μg/mL	In vitro incubation	3 hours	

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. F-627 is a recombinant fusion protein with a molecular weight of 95000 Daltons. Results from preclinical studies showed that the toxicity of F-627 was due to the exaggerated pharmacology effects, which is closely associated with the dose (expressed in mg/kg) in various different species. Based on the guideline issued by the FDA in 2005, the maximum safe starting dose of F-627 was calculated to be 67.5 μg/kg using mg/kg conversion, which is one-tenth of the NOAEL in cynomolgus monkeys. Based on the dose in the phase I clinical trial conducted in Australia, 80 μg/kg is a safe starting dose.

1.1.5 Preclinical pharmacodynamic 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 proliferation effects of F-627 on M-NFS-60 was 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 with filgrastim (Neupogen, Amgen) and pegfilgrastim (Neulasta, Amgen; Neulastim, Roche), 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 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-627 100 μg /kg in mice showed that, phosphorylated STAT3 levels in bone marrow cells increased 17 folds compared to baseline levels. Peak ANC in peripheral blood appeared at 48 hr after the dose, and recovered to normal levels at 72 hr. 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 cynomolgus monkey models with cyclophosphamide-induced neutropenia, F-627 not only shorten the recovery time of ANC, but also reduced the ANC decrease compared to filgrastim (rhG-CSF) and pegfilgrastim (Neulasta), thereby preventing the occurrence of severe neutropenia. F-627 demonstrated superior pharmacodynamic effects over the same dose (60 μg/kg) of pegfilgrastim.

1.1.6 Previous clinical studies

The phase I clinical trial was conducted at the Alfred Medical Center in Melbourne, Australia. The clinical study protocol was approved by the Ethics Committee of the hospital on May 4, 2010, and the first subject received F-627 treatment on Jun. 1, 2010.

The starting dose of this study was 30 μg/kg, and the dose was escalated sequentially to 60, 120, 240, and 360 μg/kg. In each dose cohort, 6 healthy adult male subjects were enrolled, which means totally 30 subjects in five cohorts. One to three Asian subjects were recruited to each dose cohort except for the 360 μg/kg cohort. Each subject received a single-dose of F-627 by subcutaneous injection. Blood samples were then collected at multiple time points in the 14-day follow-up visits to evaluate the pharmacokinetics, pharmacodynamics (including WBC, ANC, and CD34 analysis), and safety of the drug. The starting dose of F-627 was 30 μg/kg. Dose escalation was conducted only after it was confirmed to be safe by the principal investigators and medical experts of the sponsor.

All subjects completed dose administration. F-627 subcutaneous injection was well-tolerated. There were no serious adverse events (SAEs) or deaths among the 30 subjects. All adverse events are summarized in Table 30-3. No abnormalities were observed in the vital signs, electrocardiograms (ECGs), abdominal ultrasound, serum biomarkers and urinalysis parameters of subjects in all dose cohorts. The most common adverse events were manifested as musculoskeletal disorders, including mild to moderate muscle soreness, bone pain and lumbago.

Table 30-3. Summary of adverse events in the phase I clinical trial.

Adverse Events	30 μg/kg	60 μg/kg	120 μg/kg	240 μg/kg	360 μg/kg	Total Number	Description
Headache	1	2	1	1	2	7	2 moderate events
Injection-Site Pain	2			0		2	1 moderate events
Bone Pain		2		3		5	3 moderate events

Adverse Events	30 μg/kg	60 μg/kg	120 μg/kg	240 μg/kg	360 μg/kg	Total Number	Description
Back Pain	2	1	4	4	4	15	6 moderate events
Leg Pain		1		1	2	4	3 moderate events
Abdominal Discomfort/Pain		1		1		2	1 moderate events
Dry Skin		1				1	1 moderate events
Hunger			1			1	
Urinary Tract Infection				1		2	
Diarrhea				2		2	
Feeling Abnormal				1		1	
Tiredness					1	1	
Respiratory Tract Irritation				1		1	
Photophobia				1		1	

All moderate adverse events were noted, others were mild events.

Results are shown in Table 30-4. The 5 dose cohorts, 30, 60, 120, 240, and 360 μg/kg, demonstrated dose-dependent pharmacodynamics, i.e., WBC, ANC, and CD34 count increased with increasing dose. The lowest dose cohort (30 μg/kg) demonstrated significant ANC elevation. The time to initial onset of effect was 4 hours for all dose cohorts. In the lowest dose cohort, ANC at 4 hours after administration (5.05 ± 2.00) was significantly higher than that before administration (2.79 ± 0.40, p = 0.02). T_{max} of ANC was 36 hours for two low dose cohorts and 72 hours for two high dose cohorts. The peak ANC in these four dose cohorts increased 4.1, 4.4, 5.8, and 8.6 folds compared to the pre-dose ANC. CD34 peaked at 72–96 hours, respectively. CD34 reached 38.7 ± 11.7 in the 120 μg/kg cohort. Results showed a good dose-effect relationship of F-627 in healthy male subjects.

Table 30-4. Human PK parameters in phase I clinical trial.

Parameters	30 μg/kg (n=6)	60 μg/kg (n=6)	120 μg/kg (n=6)	240 μg/kg (n=6)	360 μg/kg (n=6)
C_{max} (ng/mL)	21.3(10.3)	44.6(17.7)	219.9(76.6)	759(160)	693(243)
T_{max} (hr)	8(8-16)	8(8-16)	16(16-36)	36(36)	16(16-48)
$T_{1/2}$ (hr)	43.9(9.3)	56.1(23.3)	59.3(23.5)	62.8(10.8)	71.4(27.3)
$AUC_{(0-\infty)}$ (ng·hr/mL)	720(214)	1756(673)	8374(2789)	46580(17255)	44009(18266)
CL/F (mL/hr/kg)	41.4(12.8)	36.8(14.6)	18.5(7.7)	5.7(2.0)	12.0/11.9

Figure 30-1. Semi-logarithmic plot of concentration-time (hours) after different doses of F-627 in healthy male subjects.

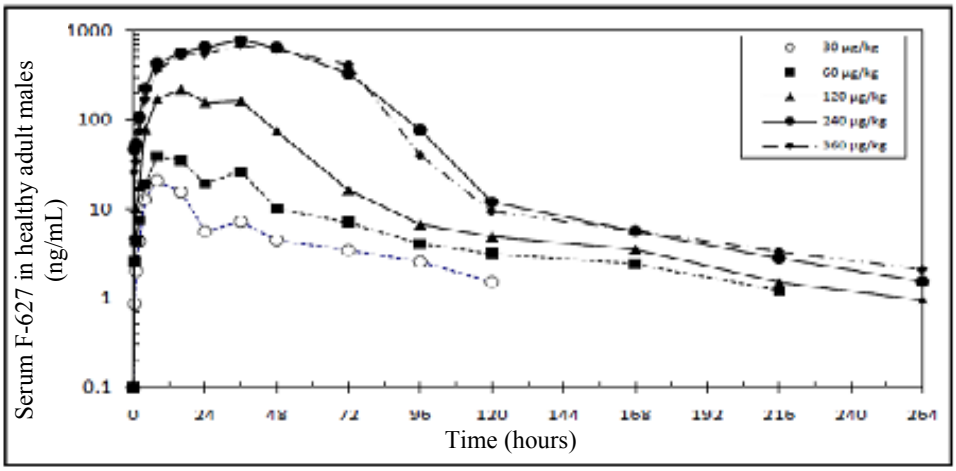
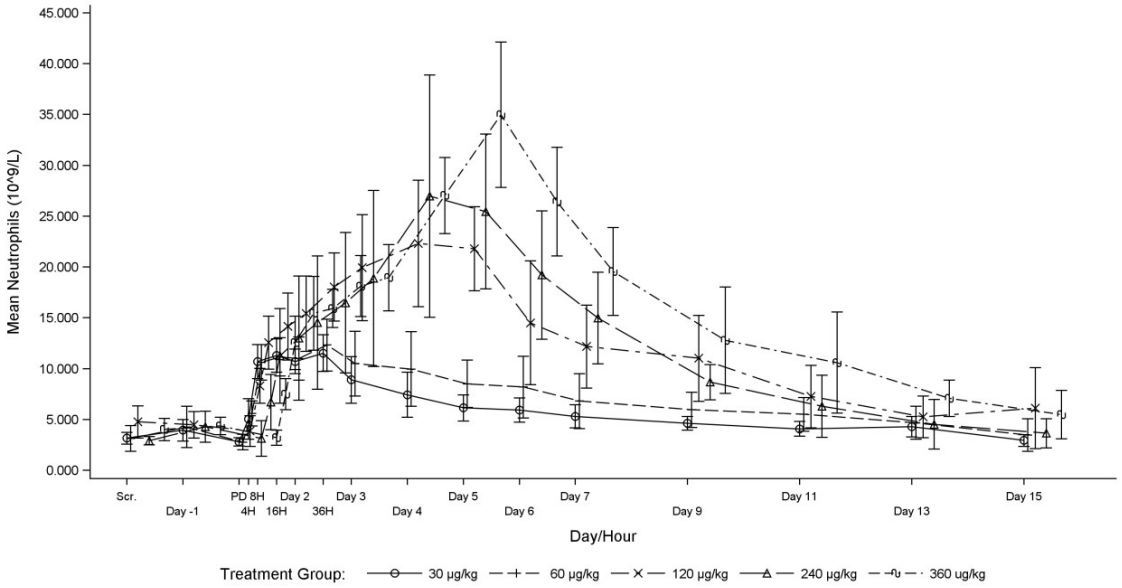


Table 30-5. Summary of pharmacodynamics in phase I clinical trial.

F-627 Dose μg/kg	ANC Elevation (T_{max}) hrs	Peak ANC (Increased Folds)	ANC Recovery hrs	CD34 T_{max} (CD34 Count)
30	4 (36)	11.52 (4.1)	144	96 (7.83 ± 2.32)
60	4 (36)	12.32 (4.4)	240	72 (21.5 ± 23.9)
120	4 (72)	22.31 (5.8)	288	72 (38.67 ± 11.71)
240	4 (72)	26.98 (8.6)	240	96 (37.83 ± 9.7)
360	5(96)	34.97(10.9)	To be supplemented	120(52.7 ± 19.0)

ANC units: 1×10^9/L; ANC recovery: ANC < 5×10^9/L; CD34 count units: 1×10^3/mL, n = 6.

Figure 30-2. Mean ANC in subjects after administration of different doses of F-627.



Clinical studies of long-acting rhG-CSF (pegfilgrastim, i.e. PEGylated G-CSF) were primarily conducted by Amgen Inc. The new drug approval documents of pegfilgrastim included two phase I clinical trials that examined the PK/PD and safety of the drug in healthy human; four phase II clinical trials that determined the effective dose of the drug in patients with different cancers, including post-chemotherapy lymphoma, lung cancer and breast cancer; and two phase III clinical trials that examined the efficacy and safety of 100 μg/kg pegfilgrastim. Pegfilgrastim was approved for marketing by the FDA in 2002 and is mainly used in Europe, U.S. and other western countries. Pegfilgrastim is a first-line supportive therapy for post-chemotherapy cancer patients. It is used to treat neutropenia caused by radiation therapy and chemotherapy.

There has been a substantial number of clinical studies of pegfilgrastim in recent years. A total of 107 clinical studies could be found in the Pubmed database using pegfilgrastim or Neulasta as keywords for title and abstract. Long-acting pegfilgrastim is favorable for patients as it is administered once per chemotherapy cycle and its efficacy is equal to that of the short-acting Filgrastim administered daily. However, most blood cancer patients, such as those with lymphoma or acute leukemia, are in a state of severe neutropenia for a considerable period of time (2-3 weeks) even with post-chemotherapy administration of filgrastim or pegfilgrastim. Therefore, this is the medical challenge facing the application of the existing recombinant human G-CSF monomers.

The clinical side effects of long-acting Pegfilgrastim are similar with those of short-acting Filgrastim. These side effects are primarily manifested as musculoskeletal disorders including muscle soreness, bone pain, lumbago and chest pain, and gastrointestinal disorders including inappetence or increased ALT/AST in liver. Some patients may develop fever, headache, asthenia, rash and ALP/LDH elevation, and a small fraction of patients may experience shock, interstitial pneumonia, adult respiratory distress syndrome, and increased immature granulocyte count. Pegfilgrastim is prohibited for individuals with severe liver, kidney, heart and lung disorders. Pegfilgrastim is not recommended for myeloid leukemia patients without significant reduction in immature granulocytes in the bone marrow or with immature granulocytes in the peripheral blood.

1.2 Overall Evaluation of Adjuvant Measures and Efficacy of Chemotherapy for Cancer

Cancer is currently still a threat to human health and its incidence rate continues to increase (Nature Review Cancer, 2006, vol 6: 63-74). In 2002, there were 10.9 million new cancer patients worldwide. The number of new cancer patients worldwide is estimated to reach 16.5 million in 2020 and 27.0 million in 2050. According to the statistics from the Ministry of Health of the PRC, the incidence rate of cancer had increased to 127 in 100000 people in the 1990s. In recent years, the number of new cancer patients per year has reached 1.6–1.7 million, totaling about 4.5 million cancer patients. In terms of cancer treatment, chemotherapeutic and cytotoxic agents are still first-line therapy. Some new small molecule targeted drugs still cannot be used as first-line drugs for curing cancer. Therefore, there is still much to be done for the adjuvant therapy of chemotherapy. Although first- and second-generation G-CSF has partially shortened the duration of chemotherapy-induced neutropenia, how to alleviate neutropenia and shorten the duration of severe neutropenia are still the challenges in the treatment of chemotherapy-induced severe neutropenia. The development and study of F-627 have provided theoretical and practical feasibility for the treatment of this problem. As an independent global innovation, F-627 is expected to become the best adjuvant therapy for enhancing the efficacy of chemotherapy drugs in cancer patients.

2. STUDY OBJECTIVES

2.1 Primary Objective

To evaluate the safety and tolerability of recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection (F-627) in treatment of female postoperative breast cancer patients who require adjuvant chemotherapy in a single-center, open-label, single-dose and repeated-dose, dose-escalation phase I clinical trial.

2.2 Secondary Objectives

1) To evaluate the pharmacokinetics (PK) of F-627 by determining the serum drug concentrations of F-627 at different time points after dosing;
2) To evaluate the pharmacodynamics of F-627 by analyzing the relationship between serum drug concentrations of F-627 and neutrophil counts in blood at different time points after dosing, and to provide a recommended dose for phase II clinical trial.

2.3 Exploratory Objectives

1) To observe the number of days in which absolute neutrophil count is less than 0.5×10^9/L in cycle 1 of chemotherapy, the average time of ANC recovery, and the number of days in which absolute neutrophil count is less than 1×10^9/L, and to provide insights for phase II clinical trial.
2) To evaluate the potential immunogenicity of F-627 by testing anti-F-627 antibodies (IgG and IgM) in serum.

3. STUDY PLAN AND METHODOLOGY

3.1 Overall Study Design and Schedule of Study Procedures

This is a single-center, open-label, dose-escalation, single-dose and repeated-dose phase I clinical trial in treatment of female postoperative breast cancer patients who require adjuvant chemotherapy. Eligible patients are those who have not received chemotherapy. The adjuvant chemotherapy regimen for this trial is EC→P or EC→T, 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 all 4 cycles, subjects will receive 4 subsequent cycles of sequential chemotherapy (either paclitaxel or docetaxel) and supportive care according to routine clinical practice. The investigator must ensure that the first cycle of treatment follows the recommended chemotherapy regimen; in chemotherapy cycles 2–4, treatment regimen and dose are permitted to be individualized based on subject conditions. Dose delay and one dose reduction due to toxicities other than myelotoxicity (such as cardiotoxicity) are permitted in this trial. If the subject requires a second dose reduction, the investigator and sponsor must decide together whether the subject should continue the treatment with study drug.

Subjects who have signed the informed consent form and have passed the screening are enrolled in the trial.

This study includes 3 dose cohorts, 80, 240, and 320 μg/kg, each of which will enroll 6 subjects sequentially. Subjects in each cohort will receive a single-dose of F-627 by subcutaneous injection approximately 48 hours after the completion of chemotherapy. Blood samples are then collected at multiple time points in the subsequent follow-up visits to evaluate the pharmacokinetics, pharmacodynamics, and safety of the drug. If dose-limiting toxicities (DLT, see Section 3.4) are not observed before the start of cycle 2, then the same dose of F-627 is to be given at approximately 48 hours after each chemotherapy in cycles 2–4.

Only after 6 subjects in each cohort have completed the treatment and observation of the first cycle will the sponsor and investigator determine whether to proceed to the next higher dose based on the safety evaluation.

Schedule of study procedures is detailed in Table 30-6.

Table 30-6. Schedule of F-627 study procedures.

	1–15 Days Before Enrollment	Day 1, Day 22, Day 43, and Day 64 of the Study[6]	Day 3, Day 24, Day 45, and Day 66 of the Study[1]	Cycle 1 of Chemotherapy (Day 3–Day 21)[4]	Cycles 2–4 of Chemotherapy (Day 3–Day 21)[5]	End of Study (Day 84)[7]
Signing Informed Consent Form	X					
History of Cancer	X					
Complete Physical Examination	X					X
Physical Examination		X	X			
Abdominal Ultrasound[2]	X	X				X
Chemotherapy		X				
Urinalysis[2]	X	X				X
Administration[1]			X			
12-Lead ECG[3]	X					X
Color Doppler Echocardiography	X					
Chest X-Ray	X					X
Height and Weight[2]	X	X				
Body Temperature	X	X	X	X	X	X
Routine Blood Test (including ANC)	X	X	X	X	X	X
Clinical Chemistry[2]	X	X				X
Serum Pregnancy Test	X					X
Blood Pressure and Heart Rate	X	X				X
Pharmacokinetics[8]			X	X	X	
Serum Antibody[9]		X	X	X	X	X

	1–15 Days Before Enrollment	Day 1, Day 22, Day 43, and Day 64 of the Study[6]	Day 3, Day 24, Day 45, and Day 66 of the Study[1]	Cycle 1 of Chemotherapy (Day 3–Day 21)[4]	Cycles 2–4 of Chemotherapy (Day 3–Day 21)[5]	End of Study (Day 84)[7]
AEs and Combined Medications[9]		X	X	X	X	X

1. Three dose cohorts for F-627 (80 μg/kg, 240 μg/kg and 320 μg/kg);
2. Examination should be completed upon enrollment and the start of each chemotherapy cycle. Body height is measured upon enrollment only, while body weight is measured on day 1 of each cycle; for abdominal ultrasound, a retest on day 1 of cycle 1 is not required and the baseline result can be used; for routine blood test and clinical chemistry before the start of cycle 1, results of the tests performed within 7 days before cycle 1 are acceptable;
3. 12-lead ECG should be repeated at the last visit of the study;
4. For chemotherapy cycle 1, starting from day 3, oral temperature measurement and routine blood test will be performed daily until ANC recovers to > 1.0×10^9/L from nadir, and once every 3 days thereafter until the next cycle;
5. For chemotherapy cycles 2–4 (day 3–day 21 of each chemotherapy cycle, i.e., day 24–day 84 of the study), starting on each day 3 of cycles 2–4, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to > 1.0×10^9/L, and once every 3 days thereafter until the next cycle;
6. Only when ANC of a subject recovers to > 1.0×10^9/L as judged by the investigator, can the next cycle start;
7. The last visit is on day 84, and the subjects should complete a follow-up visit by telephone on day 114 (30 days after the last visit);
8. In cycle 1 and cycle 3, blood samples are collected for PK at time points shown in Table 30-8;
9. Blood samples will be collected for serum antibody assays during screening or before treatment with the drugs, as well as on days 8, 13, and 21 of each cycle.

Remark: All laboratory measurements will be performed at a central laboratory assigned by the investigator. In case of any toxicities related to chemotherapy, subsequent treatment can be modified according to common diagnosis and treatment practice in the judgment of the investigator.

3.2 Evidence for Dose Determination

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. F-627 is a recombinant fusion protein with a molecular weight of 95000 Daltons. Results from preclinical studies showed that the toxicity of F-627 was due to the amplification of pharmacodynamics, which is closely associated with the dose (expressed in mg/kg) in various different species. Based on the guideline issued by the FDA in 2005, the maximum safe starting dose of F-627 was calculated to be 67.5 μg/kg using mg/kg conversion, which is one-tenth of the NOAEL in cynomolgus monkeys. Based on the dose for healthy volunteers in the phase I clinical trial, 80 μg/kg is a safe starting dose.

The pharmacokinetic parameters in cynomolgus monkeys, such as $T_{1/2}$, AUC, CL and MRT, were comparable between F-627 and pegfilgrastim. In the 3-month repeat dosing toxicity studies of pegfilgrastim and F-627 in rats and cynomolgus monkeys based on subcutaneous injection, the no-observed-adverse-effect-levels (NOAELs) of drugs in rats were comparable to those in cynomolgus monkeys. In addition, the phase I clinical trial involving healthy volunteers used an F-627 dose that is less than 5% of the NOAEL in monkeys (Table 30-6). F-627 demonstrated good safety and tolerability in all five dose cohorts (30, 60, 120, 240 and 360 μg/kg). For F-627, 240 μg/kg was equivalent to 96 μg/kg of pegfilgrastim (calculated by the molarity of G-CSF). The phase I of this study plans to include three dose cohorts, namely 80, 240 and 320 μg/kg. Firstly, all doses should not exceed the maximum dose in the phase I clinical trial. Secondly, the G-CSF molarity of 240 μg/kg F-627 is comparable to that of 100 μg/kg pegfilgrastim. Finally, 60 μg/kg of F-627 showed pharmacodynamics

superior than 150 μg/kg in the cynomolgus monkey models of chemotherapy-induced neutropenia. Therefore, using 80 μg/kg in the low-dose cohort will ensure that the drug may be effective for the subjects. The highest dose (320 μg/kg) for subjects is slightly lower than the maximum dose (360 μg/kg) tested in healthy subjects.

Table 30-7. Rationale for F-627 doses in clinical trials.

	Pegfilgrastim	F-627
Repeat Dosing Toxicity Study in Rats	100, 300, and 1000μg/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
Starting Dose of Healthy Subjects in Phase I Clinical Trial	30 μg/kg	30 μg/kg
Dose of Healthy Subjects in Phase I Clinical Trial	30, 60, 100, 300 μg/kg	30, 60, 120, 240, 360 μg/kg
Post-Chemotherapy Dose for Breast Cancer in Clinical Trial	60, 100, 300 μg/kg	Planned: 80, 240 and 320 μg/kg
Recommended Dose for Clinical Trials	100 μg/kg	TBD

3.3 Single Dose and Repeated Dose

This study includes 3 dose cohorts, 80, 240, and 320 μg/kg, each of which will enroll 6 patients with breast cancer sequentially receiving adjuvant chemotherapy. Subjects in each cohort will receive a single-dose of F-627 by subcutaneous injection approximately 48 hours after the completion of chemotherapy. Blood samples are then collected at multiple time points in the subsequent follow-up visits to evaluate the pharmacokinetics, pharmacodynamics, and safety of the drug. If dose-limiting toxicities (DLT, see Section 3.4) are not observed before the start of cycle 2, then the same dose of F-627 is to be given at approximately 48 hours after each chemotherapy in cycles 2–4.

3.4 Dose Escalation

Doses that will be used in this study are 80, 240 and 320 μg/kg, and 6 subjects will be enrolled in each dose cohort. The starting dose is 80 μg/kg. Only after 6 subjects have completed the treatment and observation of the first cycle will the sponsor and investigator determine whether to proceed to the next higher dose based on the safety evaluation. Likewise, the 240 μg/kg cohort will enroll 6 subjects, and only after 6 subjects have completed the treatment and observation of the first cycle will the sponsor and investigator determine whether to proceed to the next higher dose based on the safety evaluation. The 320 μg/kg cohort will enroll 6 subjects, but dose escalation will not be conducted after all 6 subjects have completed the treatment and observation.

Dose Cohort	Dose	Number of Subjects
1	80 μg/kg	6

2	240 μg/kg	6
3	320 μg/kg	6

Dose escalation should be stopped in each dose cohort if 2 dose-limiting toxicities (DLT, see Section 3.5 for definition) are observed in subjects in cycle 1 of chemotherapy

3.5 Dose-Limiting Toxicity (DLT)

Dose-limiting toxicity (DLT) refers to an intolerable toxicity that is observed in the treatment with investigational drug and limits the further dose escalations. DLT is defined as any grade 3 or greater adverse event related to the investigational drug that observed in cycle 1 (21 days). Adverse events will be assessed according to NCI CTCAE V4.03 criteria.

After 6 subjects in each F-627 dose cohort have completed the treatment and observation of first cycle, the trial may proceed into the next dose cohort only if less than 2 subjects develop DLT. If a grade 3 or greater adverse event related to the investigational drug is observed in cycles 2–4, then the investigator and sponsor will determine together whether the adverse event would affect further dose escalations.

3.6 Risk, Benefit and Ethical Evaluations

The rhG-CSF-Fc fusion protein is a long-acting rhG-CSF expressed in CHO cells. Each molecule of rhG-CSF-Fc fusion protein contains two G-CSF dimer molecules, which may overcome the weak bioactivity of pegfilgrastim and produce a stronger receptor activation signal, thereby accelerating the differentiation and proliferation of neutrophils in bone marrow. Also, preclinical studies have shown that the pharmacokinetic and pharmacodynamic properties of rhG-CSF-Fc is different from or superior to pegfilgrastim, and therefore it decreases the severity of neutropenia and reduces the duration of severe neutropenia in cancer patients after chemotherapy. For the phase I clinical trial, patients with breast cancer will be enrolled as subjects to evaluate the safety, tolerability and pharmacodynamic properties of F-627. Phase I clinical trial of pegfilgrastim showed that subcutaneous injection of 30, 60, 100 and 300 μg/kg of pegfilgrastim resulted in no significant toxicity. The dose of F-627 will also be controlled within this range to ensure the safety of the subjects. Phase I clinical trial of F-627 in Australia already demonstrated that no serious adverse event is observed within this dose range and subjects had good treatment compliance.

In this study, the safety of the subjects will be closely monitored, including clinical symptoms, vital signs, routine blood test, clinical chemistry, urinalysis and any adverse event. Once an adverse event is observed, it will be handled in accordance with the national regulations. Safety evaluation of the phase I clinical trial on healthy subjects showed that adverse events observed under the above doses were all within controllable range. Emergency medical events and handling procedures are described in Section 11 of this document.

4. STUDY POPULATION

4.1 Study Population and Number of Subjects

Female postoperative patients with breast cancer who receive adjuvant chemotherapy will be enrolled in this study. Eligible subject should be chemotherapy naïve patient.

The selection of chemotherapy drug is one of the key considerations in the design of this protocol. Among the adjuvant chemotherapy regimens that are recommended by the NCCN 2010 guideline for breast cancer, the preferred regimens include TAC, dose-dense AC → dose-dense paclitaxel, AC → paclitaxel, TC, EC, and AC. At present, AC/EC→P or AC/EC→T are the commonly used regimens in China.

The adjuvant chemotherapy regimen for this trial is EC→P or EC→T, that is: 100 mg/m^2 epirubicin i.v. + 600 mg/m^2 cyclophosphamide i.v. on day 1, repeat cycle every 21 days for 4 cycles. After completing the evaluation for all 4 cycles, subjects will receive 4 subsequent cycles of sequential chemotherapy (either paclitaxel or docetaxel) and supportive care according to routine clinical practice.

The investigator must ensure that the first cycle of treatment follows the recommended chemotherapy regimen; in chemotherapy cycles 2–4, treatment regimen and dose are permitted to be individualized based on subject conditions. Dose delay and one dose reduction due to toxicities other than myelotoxicity (such as cardiotoxicity) are permitted in this trial. If the subject requires a second dose reduction, the investigator and sponsor must decide together whether the subject should continue the treatment with study drug.

This study includes 3 dose cohorts, 80, 240, and 320 μg/kg, each of which will enroll 6 subjects, totaling 18 subjects.

4.2 Inclusion Criteria

1) 18-75 years old;
2) Female postoperative breast cancer patients who require adjuvant chemotherapy, and are planned to receive 4 cycles of EC chemotherapy;
3) ECOG performance status of 0-1;
4) Absolute neutrophil count (ANC) ≥ 2.0 × 10^9/L, hemoglobin (Hb) ≥ 11.5 g/dL, and platelet (PLT) ≥ 100 × 10^9/L prior to chemotherapy;
5) Hepatic and renal function within the normal range;
6) Left ventricular ejection fraction greater than 50%
7) Willing to sign the informed consent form and able to comply with protocol requirements;

4.3 Exclusion Criteria

Subjects who meet any of the following must be excluded from this study:

1) Women in pregnancy or breastfeeding; women of child–bearing potential have a positive pregnancy

test result prior to the first dose;

2) Life expectancy less than 12 months;
3) Radiation therapy within 4 weeks prior to enrollment;
4) Patients with breast cancer who have received neoadjuvant chemotherapy before radical mastectomy;
5) Prior bone marrow or stem cell transplant;
6) With other malignant tumors other than breast cancer;
7) Have received G-CSF treatment within 6 weeks prior to enrollment;
8) Diagnosed with acute congestive heart failure, cardiomyopathy, or myocardial infarction by clinical diagnosis, ECG or other approaches
9) With any disease that may cause splenomegaly;
10) With acute infection, chronic active Hepatitis B within 1 year (unless patients tested negative for HBsAg prior to enrollment) or Hepatitis C;
11) History of tuberculosis (TB); history of TB exposure, unless negative for tuberculin test; TB patients undergoing treatment; or suspected TB evaluated by chest x-ray;
12) Known HIV positive or AIDS;
13) With sickle cell anemia;
14) With alcohol or drug abuse that may affect the compliance with the study;
15) With known hypersensitivity to E. coli derived proteins, G-CSF, or excipients;
16) Has received any other study drug within 4 weeks prior to enrollment;
17) Patients with diseases or symptoms unsuitable for participating in the trial based on the investigator's judgment;

4.4 Criteria for Terminating Study

1) Incidence rate and severity of serious adverse events (SAEs) indicate the study should be terminated early as determined by the investigators and the sponsor;
2) The dose-limiting toxicity of the subjects does not recover or cannot be relieved;
3) The investigators question the safety of the drug and believe that the continuation of the study may pose serious risks to the subjects;
4) The maximum dose set in the clinical trial has been reached;
5) Data fraud, or inaccurate/incomplete collection of data;

4.5 Criteria for Subject Withdrawal

Subjects will prematurely withdraw from the study with a written explanation when the following conditions occur.

1) Subjects voluntarily withdraw during the study;

2) The investigator considers that withdrawal is for the best interest of the subject;
3) The investigator or subject believes that continuing the trial may result in intolerable adverse events;
4) Complications or worsening co-morbidities affecting subject's participation occur;
5) Safety issues of the study drug in the absence of data, subject will be exposed to potential risks if continues the participation in the study;
6) When a safety concern regarding the study drug arises, but data is yet unknown, resulting in potential risks if subjects continue the trial;

4.6 Drop-Out Criteria

Determination of drop-outs: Eligible subjects who have signed the informed consent form and been enrolled have the right to withdraw from the study at any time. Subjects who do not complete the entire observation are considered drop-outs regardless of the time or reason of the withdrawal. A replacement should be implemented immediately according to the original plan when a drop-out occurs. Blood samples from drop-out subjects should be retained and tested and processed by the sponsor.

4.7 Withdrawal-Related Procedures and Handling

The investigator should ask the withdrawn subject for the reason of withdrawal and whether there is an adverse event, and record the length of treatment and dose. If possible, the investigator should perform corresponding observation and evaluation of the withdrawn subject and conduct a follow-up for adverse events within 30 days of the last dosing. If a subject withdraws from the study due to suspected grade 2, 3 or 4 infection according to WHO risk classification criteria, then his/her biological samples must not be sent to a laboratory, and instead, should be destructed as per operating practice of the study site.

5. INVESTIGATIONAL DRUG AND TREATMENT

5.1 Source

F-627 is provided by Generon (Shanghai) Corporation Ltd.

5.2 Strength and Expiration Date

The recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection (F-627) is securely packaged and sealed by Generon (Shanghai) Corporation Ltd.

Dosage Form	Strength	Formulation		Mode of Administration	Expiration Date
Lyophilized Powder for Injection	1 mg	F-627 (rhG-CSF-Fc) Sucrose Mannitol Polysorbate 80 Sodium phosphate dibasic Sodium phosphate monobasic	1.0 mg 20.0 mg 50.0 mg 0.05 mg 0.92 mg 0.42 mg	Subcutaneous injection	2 years (tentative)
Lyophilized Powder for Injection	5 mg	F-627 (rhG-CSF-Fc) Sucrose Mannitol Polysorbate 80 Sodium phosphate dibasic Sodium phosphate monobasic	5.0 mg 20.0 mg 50.0 mg 0.05 mg 0.92 mg 0.42 mg	Subcutaneous injection	2 years (tentative)

5.3 Labeling

The recombinant human granulocyte colony stimulating factor-Fc fusion protein for injection (F-627) is stored as lyophilized powder in tubular vials (1.0 mg/2 mL vial and 5.0 mg/2 mL vial). The drug label includes the following information: investigational drug name, study number, study protocol number, storage conditions, contents, mode of administration, batch number, expiration date, and the standard notes "For clinical trials only" and "Keep out of reach of children".

5.4 Storage

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.

5.5 Drug Preparation

The pharmacist is responsible for preparing the drugs and the nurse is responsible for reviewing. During drug preparation, inject 0.5 mL of water for injection slowly into the vial along the wall, let the vial stand for 1–2 minutes, and perform subcutaneous injection after the powder is completely dissolved. The injection should be used within 1 hour after preparation. The recommended concentration for F-627 is 2.0 mg/mL. The drug is generally injected in the morning (08:00 ± 1:00). The subject should remain in a sitting or walking position within two hours after the injection.

5.6 Administration and Precautions

Subjects must sign the informed consent forms prior to enrollment. After being screened and confirmed to be enrolled, subjects will be numbered in strict order. If a subject withdraws from the study, the same subject number must not be reused. Subjects who have withdrawn are not allowed to participate in this study again. According to the study protocol, subjects are allocated to different dose cohorts and will enter the study in the order of enrollment.

The investigator should be present during administration and within 1 hour after administration to provide guidance and supervision, and be prepared to administer first aid or emergency treatment whenever needed. The specific time and date of dosing, and the drug labels should be documented in the subject's source data files and case report forms.

Subjects will receive a chemotherapy of EC on day 1 of each cycle, and a subcutaneous injection of F-627 on day 3, that is, 48 ± 2 hours after the start of chemotherapy. During the treatment, subjects should avoid strenuous physical activity or long periods of bed rest, and keep relaxed.

5.7 Salvage Therapy

In the study, an approved G-CSF treatment should be given to the patient as salvage therapy in the case of febrile neutropenia (defined as ANC < 1.0×10^9/L; a single measurement of body temperature > 38.3 °C or a fever ≥ 38.0 °C lasting for longer than 1 hour; note: body temperature measurement is based on oral temperature or equivalent armpit/rectal temperature) or grade 4 neutropenia for longer than 3 days. The sponsor recommends GRAN® (filgrastim) at a dose of 5 μg/kg/day of G-CSF, once daily for ≤ 2 weeks or until neutrophil count recovers to 1.0×10^9/L.

5.8 Drug Management

Registration and recording of the investigational drug should be in the charge of designated personnel. The study site should establish a set of systematic operating procedures to ensure that the drugs are received by designated personnel, the distribution of the drugs is accurately documented, and the drugs are used and stored properly.

1) Drug registration form:
   a. The type of drug received from the sponsor;
   b. Dosage form and strength;
   c. Batch number and expiration date;
   d. The dosage form, dose, quantity, and date of drug dispensed to the investigator (signed by both dispensers and recipients).
2) Individual drug administration record: subject name, package number, administration time, dose and mode of administration, signature of operator, and return of packaging.
3) Detailed record of drug administration, loss, scattering, and misuse.

4) Unused drugs should be stored in accordance with storage requirements. Storage conditions should be checked regularly by the same designated personnel.
5) After the study is completed, all remaining drugs should be returned and recorded, and be disposed according to the GCP by the site together with the sponsor.

5.9 Principles and Methods for Treatment of Drug Toxicities

If a subject develops a mild drug toxicity after receiving F-627 injection, symptoms should be monitored with the treatment continued. Intervention is usually not required. Toxicities related to chemotherapy should be handled according to clinical guidelines and the prescribing information of the chemotherapy drugs. Serious or life-threatening symptoms must be treated immediately, and the clinical trial for this case must be terminated.

Refer to Table 30-3 for all adverse events observed in phase I clinical trial: these symptoms do not need to be treated. At present, serious adverse events have not been observed for F-627. High-risk serious adverse events for long-acting G-CSF that have been reported include:

- Acute splenomegaly: incidence rate is around 1–10%, while the likelihood of splenic rupture is less than 1 in 10000. Patients who develop abdominal pain and were found to have splenomegaly upon physical examination or ultrasonic inspection should be treated immediately. The drugs should be discontinued.
- Adult respiratory distress syndrome (ARDS): Rare cases have been reported with clinical use of long-acting G-CSF. Treat immediately and discontinue the drugs if present.

5.10 Treatment Compliance

Administer according to subject's dosage allocation under the guidance and supervision of the investigator. The actual time and date of administration should be accurately documented for each subject. Any protocol violations should be documented in subject's source data file and case report form.

6. EVALUATIONS AND ENDPOINTS

6.1 Evaluations During Screening

Prior to enrollment, the evaluation carried out within 2 weeks before dose administration (1–14 days before enrollment) includes:

1) Record of subject demographics: date of birth, gender, ethnicity, etc.;
2) Medical history collection, including history of cancer and history of chemotherapy;
3) Record of concomitant diseases and combined medication, surgical history, etc.;
4) Record of pathological diagnosis confirmed in tumor histology or cytology;

5) Tumor staging (TNM);
   - ✧ Complete physical examination, height, weight, vital signs (temperature, pulse, respiratory rate, blood pressure), and heart rate;
6) ECOG PS score, weight evaluation;
7) Hematologic examination: 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.;
8) Clinical chemistry: including total protein (TP), albumin (ALB), globulin (G), blood glucose, blood urea nitrogen, creatinine, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), pancreatic amylase, total bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), Ca, P, Mg, K, Na, Cl, etc.;
9) Urinalysis: including pH, specific gravity, protein, cast, blood cell, urine glucose, ketones, etc.;
10) 12-lead ECG;
11) Color doppler echocardiography
12) Pregnancy test within 7 days before dose administration;
13) Chest x-ray (PA and lateral);
14) Signing informed consent form;
15) Abdominal ultrasound (liver, gallbladder, spleen, kidney, and pancreas)

Note: Only results of routine blood test and clinical chemistry conducted within 1 week before enrollment are accepted.

6.2 Clinical Evaluations During and After F-627 Treatment

Evaluations on day 1 of each chemotherapy cycle (day 1, day 22, day 43, and day 64 of the study) include:

1) Abdominal ultrasound;
2) Chemotherapy;
3) Urinalysis;
4) Height, weight;
5) Body temperature;
6) Routine blood test (including ANC)
7) Clinical chemistry;
8) Blood pressure and heart rate;
9) Serum antibody assay (IgG and IgM)
10) Adverse events and combined medication;

Evaluations on day 3 of each chemotherapy cycle (day 3, day 24, day 45, and day 66 of the study) include:

1) Physical examination
2) Investigational Drug;
3) Temperature;
4) Routine blood test (including ANC);
5) Adverse events and combined medication;

According to different time in the chemotherapy cycle, for cycle 1 (days 1–21 of both the cycle and the study), starting from day 3, oral temperature measurement and routine blood test will be performed daily until ANC recovers from nadir to $> 1.0 \times 10^9$/L, and once every 3 days thereafter until the next cycle. Evaluations include:

1) Temperature;
2) Routine blood test (including ANC);
3) Adverse events and combined medication;

For chemotherapy cycles 2–4 (days 3–21 of each chemotherapy cycle, i.e., days 23–84 of the study), starting from day 2, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to $> 1.0 \times 10^9$/L, and once every 3 days thereafter until the next cycle. Evaluations include:

1) Physical examination;
2) Temperature;
3) Routine blood test (including ANC);
4) Serum antibody assay (IgG and IgM);
5) Adverse events and combined medication

Evaluations upon the end (day 84) or withdrawal include:

1) Physical examination;
2) Abdominal ultrasound;
3) Urinalysis;
4) 12-lead ECG;
5) Chest x-ray;
6) Temperature;
7) Routine blood test (including ANC)
8) Clinical chemistry;
9) Serum pregnancy test;
10) Blood pressure and heart rate;

11) Antibody test (IgG and IgM);
12) Adverse events and combined medication

Subjects who withdraw from the study should be followed for adverse events 30 days after the last dose.

The subjects should complete telephone visits (on day 114, i.e., 30 days after the last visits).

6.3 Pharmacokinetic (PK) Evaluations

6.3.1 Determination and analysis of PK serum samples

The concentrations of drugs and metabolites in blood samples will be determined by the validated central laboratory (Covance Pharmaceutical Research and Development (Shanghai) Co., Ltd.). The specific method of determining serum drug concentrations will be decided by the laboratory and will be described in detail in the clinical study report.

6.3.2 Collection, handling, and storage of PK blood samples

According to the time points specified in the study protocol, baseline blood samples are collected within 1 hour before dose administration. Refer to 30-8 for the blood collection interval. The actual blood collection time should be documented after each intravenous blood sampling. The sampling date and collection tube number should be documented in the case report form (CRF). The collect tube label should include the following information: study number, subject initials, cycle number, day, sampling time (hour), etc. Each time a total of 2.5 mL of blood is collected, transferred to a labeled serum separator tube, let stand for about 30 minutes, and centrifuged at 1000 g for 15 minutes at room temperature. Aliquot the serum into two clean 1.5 mL EP tubes (approximately 0.3 mL of serum each), close the lid tightly and immediately transfer to ≤ –70 °C for storage. The entire sampling and serum collection process must be completed within 60 minutes. Samples must be transferred in a constant temperature box with dry ice.

6.3.3 Blood sampling of single-dose and repeated-dose PK studies

PK analysis of F-627 in the phase I clinical trial showed that mean time to peak was 30–36 hours and plasma elimination half-life was 46–72 hours, longer in the high-dose cohort. For example, in the 240 μg/kg cohort, T_{max} was 36 hours, $T_{1/2}$ was 62.8 hours, MRT was 43.5 ± 6.0 hours, C_{max} was 758 ± 160 ng/mL, AUC was 46580 ± 17255 ng/mL*hr, and CL_z/F was 5.72 ± 2.0 mL/hr/kg.

Taking into account the above results from animal PK studies, this phase I clinical trial plans to collect blood samples at different time points following single-dose and repeated-dose administration. See Table 30-8 for detailed blood sampling time.

Table 30-8. Blood sampling time of single-dose and repeated-dose PK studies.

Study Process	Days of Study	Days of Dosing	Blood Sampling Time Point of Cycle 1 (hours: minutes)	Blood Sample Number	Blood Sampling Time Point of Cycle 3 (hours: minutes)	Blood Sample Number
				F-627 PK Study (2.5 mL)		F-627 PK Study (2.5 mL)
Study Process	**3**	1	–01:00±0.1:00	PK 1	00:00±0.1:00	PK 1
			02:00±0.3:00	PK 2	02:00±0.3:00	PK 2
			06:00±0.5:00	PK 3	06:00±0.5:00	PK 3
			12:00±0.5:00	PK 4	12.00±0.5:00	PK 4
	4	2	24:00±0.5:00	PK 5	24:00±0.5:00	PK 5
			36:00±0.5:00	PK 6	36:00±0.5:00	PK 6
	5	3	48:00±1:00	PK 7	48:00±1:00	PK 7
	6	4	72:00±1:00	PK 8	72:00±1:00	PK 8
	7	5	96:00±1:00	PK 9	96:00±1:00	PK 9
	8	6	120:00±1:00	PK 10	120:00±1:00	PK 10
	9	7	144:00±1:00	PK 11	144:00±1:00	PK 11
	11	9	192:00±2:00	PK 12	192:00±2:00	PK 12
	13	11	240:00±2:00	PK 13	240:00±2:00	PK 13
Number of Blood Samples Collected on Each Subject				13		13
Total Volume of Blood Samples Collected on Each Subject				32.5mL		32.5 mL

6.4 Pharmacodynamic Evaluation

While evaluating the safety, tolerability and pharmacokinetic characteristics of F-627, the ANC-time profile after treatment is observed to investigate the effects of different dosages on neutrophil count and to determine a recommended dose for phase II trials.

Pharmacodynamic evaluation of F-627 is carried out at the study site. For cycle 1, starting from day 3, oral temperature measurement and routine blood test will be performed daily until ANC recovers from its nadir to a value not less than 10×10^9/L, and once every 3 days thereafter until the next cycle; for chemotherapy cycles 2–4 (days 3–21 of each chemotherapy cycle, i.e., days 24–84 of the study), starting from each day 3 of cycles 2–4, oral temperature measurement and routine blood test will be performed every other day until ANC recovers from its nadir to $> 10 \times 10^9$/L, and once every 3 days thereafter until the next cycle. Refer to Table 30-6 for specific procedures.

6.5 Safety Evaluation

Safety evaluations include all observed and recorded adverse events and serious adverse events, changes in complete physical examinations, vital signs, performance status, routine blood test, routine urinalysis, chemistry indicators, echocardiogram, and ECG.
Refer to Section 7 for the definition of adverse events, which are graded according to NCI CTCAE V4.03. The investigator should take appropriate treatment measures on the adverse events, report the event in the corresponding form of CRFs, and determine the relationship between the adverse event or

serious adverse event and the drugs. If a serious safety issue occurs during the trial, the sponsor and investigator must decide together whether to terminate the trial. The sponsor may also request to terminate the clinical trial according to the situation.

6.6 Serum Antibody Assays and Evaluations

This study will evaluate the potential immunogenicity of F-627 by testing serum anti-F-627 antibodies (IgG and IgM) in serum.

Serum antibody assays will be carried out at the laboratory of Generon (Shanghai) Corporation Ltd. Specific assay methods will be determined by the laboratory and will be described in detail in the clinical study report.

According to the study protocol, blood samples will be collected during screening (baseline value), and on days 8, 13, and 21 of each cycle. A total of 2.5 mL of blood will be collected.

Each time a total of 2.5 mL of blood is collected, transferred to a labeled serum separator tube, placed for about 30 minutes, and centrifuged at 1000 g for 15 minutes at room temperature. Aliquot the serum into two clean 1.5 mL EP tubes (approximately 0.3 mL of serum each), close the lid tightly and immediately transfer to ≤ −70 °C for storage. The entire sampling and serum collection process must be completed within 60 minutes. Samples must be transferred in a constant temperature box with dry ice. The collect tube label should include the following information: study number, subject initials, cycle number, day, sampling time (hour), etc.

7. ADVERSE EVENTS

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

7.1.1 Clinical adverse events

An AE 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 (investigational 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 CRFs. 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 guidelines to assess the severity of AEs that are not graded by NCI CTCAE:

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	The AE is life-threatening

7.1.1.2 Causality

The investigator will use "probably related", "possibly related", "unlikely related", and "unrelated" to assess the causality between adverse events and treatment. The evaluation criteria are shown in Appendix 1 (classification of causality between adverse events and drugs).

7.1.1.3 Serious adverse events

An AE meeting 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.

Death	An adverse event that causes the death (reporting of deaths resulting from progression of disease is exempted)
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, vomiting, diarrhea, influenza, or accidental injury (e.g. ankle sprains).
Important medical events requiring pharmaceutical or surgical interventions to prevent serious medical events	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 not requiring 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 drugs, interventions taken and outcome of an SAE should be included in the report.

7.1.2 Treatment and follow-up of adverse events

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

7.1.3 Abnormal values of laboratory measurements

7.1.3.1 Laboratory measurements

Results of laboratory measurement/vital signs should be recorded in the CRF. 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/vital signs should be recorded in CRFs as independent AEs if at least one of the followings are 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)
- Resulting in treatment interruption
- The investigator insists on reporting as an adverse event: If an abnormality in laboratory measurement/vital signs is associated with clinical symptoms/sign, the corresponding clinical symptom/sign should be reported as an adverse event, and the abnormal lab result or vital sign should be recorded in the case report form as supplemental information.

7.1.3.2 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 Adverse Event Management

7.2.1 Adverse event 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 investigational 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 investigational 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 adverse events and serious adverse events that occur from the signing of the informed consent form to 30 days after stopping the investigational drug should be collected, regardless of whether the event is observed by the investigator or self-reported by the subject.

7.2.2 Serious adverse event reporting (immediate)

If any clinical AEs or laboratory abnormalities that occur from the study to 30 days after the last dose are considered as SAEs, the investigator must report the SAEs to the sponsor and regulatory authorities in accordance with applicable regulations within 24 hours after learning of the event, regardless of whether interventions are given.

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

7.2.3 Special non-serious adverse event reporting

Progression of Disease

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

Lack of Treatment Efficacy

When the disease treated by the investigational drug worsens, it may not be possible to determine whether it is due to lack of treatment efficacy or an adverse event. In this case, it is generally considered to be due to lack of treatment efficacy rather than an adverse event unless the investigator believes that the exacerbation is related to the investigational drug.

Infusion Reaction

Other than reporting an "infusion reaction", symptoms and AEs associated with the investigational 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 CRFs;
- Severity of each AE;
- Symptoms that occur within 24 hours from the infusion, e.g. fever, chills, and hypotension.

Overdose

An overdose (whether accidental or intentional) must be reported according to procedures of treating the overdose, regardless of whether there are symptoms related to the overdose. All overdose-related symptoms should be reported as AEs.

7.2.4 Pregnancy

Subjects who become pregnant during the study must immediately notify the investigator. The investigational drug and chemotherapy drug must be discontinued. 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. STATISTICAL METHODS AND ANALYSIS

Descriptive statistics will be summarized for all variables obtained at various observation time points by dose cohorts, unless the protocol specifies that statistical analysis at a particular time point is not required. Overall, continuous variables (such as age) will be descriptively summarized with observed numbers, mean, median, standard deviation, minimum, and maximum. Categorical variables will be descriptively summarized with frequency and percentage based on each category. Continuous safety analysis of adverse events related to the investigational drug and other safety endpoints will be performed to determine how to achieve dose escalation.

The analysis dataset includes all enrolled subjects who have received at least one dose of the study drug. This dataset is available for all analyses. Statistical methods are detailed in the Statistical Analysis Plan.

8.1 Pharmacokinetic Evaluation

Two phases (single-dose and repeated dose); All PK parameters will be summarized using descriptive statistics by dose cohorts. The non-compartmental analysis of blood drug concentration data will be performed by the central laboratory using WinNonlin Enterprise.

For C_{max} (natural logarithm) of the single-dose and repeated-dose phase, $AUC_{(0\text{-}inf)}$, AUC_t, and AUC_{last} will be analyzed by one-way ANOVA, with dose cohort as the fixed factor. The least squares mean difference between the two dose cohorts and the 90% confidence intervals are obtained from the analysis of variance, and then the least squares geometric means ratio and the 90% confidence intervals are obtained by taking the antilog.

8.2 Pharmacodynamics Evaluation

Absolute neutrophil count (ANC) after administration in different dose cohorts, as well as the number of days of ANC less than 0.5×10^9/L, the number of days of ANC less than 1.0×10^9/L, and the time of ANC recovered to 1.0×10^9/L after the completion of chemotherapy in cycles 1 and 2–4 are observed.

8.3 Statistical Analysis of Safety Data

All adverse events are listed by patient, and coded using MedDRA as per physiologic system and standard terminology. Inferential statistics are not required for safety endpoints. Abnormal values laboratory measurements, vital signs, ECG, and other safety endpoints need to be noted.

9. DATA MANAGEMENT

This study will be monitored in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use - Good Clinical Practice (ICH-GCP). The clinical research associate will have direct access to source data and will verify the data by comparing data in case report forms with the data in the original medical records (patient's permitting the clinical research associate to directly access the data is part of the informed consent). This data verification process is an important part of ensuring the quality of the study. During this process, the investigator will be urged to correct any transcription errors and omissions. Also, the demographic data in case report forms are also considered as source data and should be verified before being transferred to the data management department.

9.1 Data Filling-In by Investigator

1) All parts of the case report forms should be carefully and detailedly filled in for subjects who signed the informed consent forms and are eligible to participate in the trial, with no blanks or omissions (blank spaces should be crossed out);
2) Data in the case report forms should be verified with medical records to ensure accuracy;
3) Case report forms are considered as source data. When making changes, the investigator should cross out incorrect data, write down the correct data, then sign and date the change;
4) Data that are significantly higher or beyond reference ranges should be verified and explained by the investigator;
5) Refer to the instructions for filling in case report forms (CRFs).

9.2 Requirement of Data Monitoring by Clinical Research Associate

1) The clinical research associate should check subjects' informed consent and screening procedures during the course of the trial;
2) Ensure that case report forms are filled out correctly and consistent with source data;
3) All errors or omissions have been corrected or noted, and signed and dated by the investigator;
4) Dose modifications, treatment changes, combined medications, and intercurrent diseases should be confirmed and documented for each subject;
5) Verify that withdrawal and loss to follow-up of enrolled subjects are explained in the case report forms;
6) Ensure that all adverse events have been documented, and serious adverse events have been documented and reported;
7) Verify whether the drugs are supplied, stored, dispensed, and returned in accordance with applicable regulations, and documented accordingly;
8) CRF must be completed for each enrolled patient;
9) CRF is filled out by the investigator and must be completed for each enrolled patient. After the completed CRF is reviewed by the clinical research associate, the original copy is handed over to the data management personnel of the statistical teaching and research section for data entry and data management, the middle copy is handed over to the sponsor for archiving, and the bottom copy is archived at the clinical pharmacological center.

9.3 Data Entry, Recording and Management

This study will use paper case report forms (pCRFs). Subject data will be clearly documented on the case report forms using blue or black ink. Correction fluid or tape must not be used. The clinical research associate of Generon (Shanghai) Corporation Ltd. will verify data during monitoring visits. The investigator should ensure that the data in the case report forms are accurate, complete, and clear. Data in the case report forms will be entered into the F-627 clinical study database and validated under the direction of data management personnel. Data in the case report forms that are missing, abnormal, or inconsistent will be presented to the investigator in data query forms and documented accordingly. The database is locked only after all queries have been resolved. The statistics department is responsible for data entry and management. The data manager will use software-compiled data entry programs for data entry and management. Double entry and proofreading should be performed by two data entry personnel independently to ensure the accuracy of data. The clinical research associate should submit any queries regarding case report forms to the investigator in data query forms. The investigator should respond and return the query as soon as possible. The medical statistician will then modify, confirm, and enter the data based on the investigator's response. The data query form can be issued again if necessary.

The revision and inquiry of the data query form should be documented. The data query form is an extension to the CRF and must be kept carefully.

9.4 Audits and Inspections

The authorized representative of Generon (Shanghai) Corporation Ltd., regulatory departments, independent ethics committee may audit or inspect the study site, including source data verification. The purpose of audits or inspections is to systematically and independently check all study-related procedures and documents to ensure that procedures have been implemented and data have been recorded, analyzed, and accurately reported in accordance with study protocol, GCP, ICH guidelines, and other laws and regulations. The investigator should immediately contact Generon (Shanghai) Corporation Ltd. when an inspection of the study site has been requested by the regulatory department.

9.5 Personnel Training

The principal investigator should maintain a study work record of study-related personnel (doctors, nurses, and other personnel). The principal investigator must ensure that all personnel have received appropriate training related to the study, and any new information related to the study has been communicated to the relevant personnel.

10. STUDY PROTOCOL REVISIONS

The study protocol and study procedures must not be modified without the consent of both the principal investigator and Generon (Shanghai) Corporation Ltd. If the study protocol must be modified, the amendments or new version of the protocol (revised protocol) must be reviewed and approved in writing by the ethics committee prior to being implemented. If applicable, it must be submitted to or approved by the local drug regulatory authorities according to local regulations.

If there is an administrative amendment, the change must be submitted to or approved in writing by the ethics committee as required. Generon (Shanghai) Corporation Ltd. and the ethics committee of the study site must be notified if amendments to the study protocol require changes to the study site's informed consent form. The application of the revised informed consent form must be approved by Generon (Shanghai) Corporation Ltd. and the ethics committee in writing.

Generon (Shanghai) Corporation Ltd. will distribute the protocol amendments and the new version of the study protocol to each principal investigator. The principal investigators will then be responsible for providing these documents to the ethics committee and other personnel at the study site.

11. STUDY MANAGEMENT

11.1 Clinical Study Agreement

The principal investigator of the study site must comply with all the terms and responsibilities of this clinical study agreement. The investigator must follow the study protocol when there are contradictions between the study protocol and the study agreement.

11.2 Study Schedule and Requirements for Early Termination

The study is scheduled to begin in December 2012 and end in October 2013. If the sponsor decides to terminate or suspend the study, the investigator and regulatory authorities must be notified in writing and the reason for early termination or suspension must be provided. The principal investigator/investigator must immediately explain the situation to subjects in this study, provide appropriate treatment, take necessary measures, and document the treatment measures in the source documents and case report forms.

12. ETHICS

12.1 Ethics of Clinical Study

The study must not be initiated before the protocol is approved by the Institutional Review Board (IRB) or ethics committee (EC). The composition of the IRB or EC must be in accordance with State Food and Drug Administration (SFDA) requirements and fulfill all duties required by the SFDA. The ethics committee approval should include comments on the review or approval, as well as the name, gender, and occupation of the committee members. The clinical research associate must first receive a copy of the ethics committee approval before distributing the drugs to the investigator. The investigator must receive the ethics committee approval before enrolling subjects.

According to applicable regulations of national regulatory authorities, in case of any amendments to the study protocol, the principal investigator is responsible for submitting to and obtaining written approval from the ethics committee for all study protocol amendments. The ethics committee must review advertisements for subject recruitment. The ethics committee must also re-approve the study protocol annually as required by local regulations. All SAEs or unexpected events that occur in the study must be reported to the EC as subject safety and the conduct of the trial may be affected. Consult the EC if the ethical aspects of the study need to be re-evaluated.

This study must be conducted in strict accordance with the requirements of SFDA "Good Clinical Practice" and the "Declaration of Helsinki".

12.2 Independent Ethics Committee (IEC)

Before the clinical study can begin, the trial protocol, informed consent form, and other materials provided to subjects must be reviewed and approved by the ethics committee. Relevant approvals must be provided to the sponsor.

12.3 Informed Consent Form (ICF)

The principal investigator of the study site must ensure that subjects receive adequate oral and written information regarding the nature, purpose, potential risks and benefits of the study, and must inform subjects that they may voluntarily withdraw from the study at any time. Subjects must be given the opportunity to ask questions and time to consider these information. The informed consent form must be signed and dated by each subject before the trial is initiated. The original copy must be kept by the investigator as part of the trial documents. A copy of the signature page is retained by the subject. The subject must be informed that the sponsor's designee may review the relevant medical records.

12.4 Privacy Protection

The informed consent form will state (or sometimes with separate documents) compliance with applicable data protection and privacy regulations. Based on this statement, subjects are required to authorize the investigator or other personnel who need to know the information to collect, use, and publish their data. The informed consent form will state that the study data are stored in a computer database and kept confidential in accordance with the applicable laws. In the database, subjects are only identified based on random number/study number/subject name initials. The informed consent form will also state that for the purpose of data verification, the sponsor's designee, regulatory authorities, and the ethics committee have direct access to hospital or medical records related to this study, including patient medical records.

13. QUALITY CONTROL OF CLINICAL TRIAL

In the study, the clinical research associate assigned by the sponsor will regularly conduct on-site monitoring visits to the study hospital to ensure that all parts of the study protocol are strictly implemented and the accuracy of study data.

The personnel participating in the study must receive uniform training, make unified records and adopt the same evaluation criteria. The investigator must fill out all parts of the CRF truthfully, detailedly, and carefully according to requirements to ensure that the contents of the case report form is authentic and reliable. The evaluation criteria for abnormalities in laboratory measurement should be based on the normal reference ranges provided by the measurer.

All observations and findings in the trial should be verified to ensure data reliability and to ensure that conclusions from the trial are derived from source data. Appropriate data management measures should be set in place during the clinical trial and data processing phases. Active measures should be taken to maintain a drop-out rate of < 20%.

14. Data Storage and Use

The investigator must agree to store all study data, including confirmation of all participating subjects (different records, such as CRFs and hospital source data, can be effectively verified), original informed consent forms of all patients, CRFs, and detailed drug dispensation records.

All data from this clinical trial are owned by Generon (Shanghai) Corporation Ltd. and must not be provided to persons unrelated to this trial in any form without the consent of the sponsor. Data from this study may be published only after the consent of the sponsor.

15. REFERENCES

1 National Comprehensive Cancer Network(NCCN), Version 2010.
2 L Roskos, B B Yang et al. Pharmacokinetic/Pharmacodynamic Modeling of Pegfilgrastim in Healthy Subjects. J. Clin Pharmacol 2006; 46: 747-757
3 F. A. Holmes, S. E. Jones, J. O'Shaughnessy et al. Comparable efficacy and safety profiles of once-per-cycle pegfilgrastim and daily injection filgrastim in chemotherapy-induced neutropenia: a multicenter dose-finding study in women with breast cancer. Ann Oncol. 2002 ;13:903-9.
4 Stephen E. Jones, Michael A. Savin et al. Phase III Trial Comparing Doxorubicin Plus Cyclophosphamide With Docetaxel Plus Cyclophosphamide As Adjuvant Therapy for Operable Breast Cancer. J Clin Oncol. 2006;24:5381-5387.
5 Martin M, Pienkowski P, Mackey J, et al. Adjuvant docetaxel for node-positive breast cancer. N Engl J Med 2005;352:2302-2313.
6 Nobuyuki Yamamoto, Ikuo Sekine, Kazuhiko Nakagawa, et al. A Pharmacokinetic and Dose Escalation Study of Pegfilgrastim (KRN 125) in Lung Cancer Patients with Chemotherapy-induced Neutropenia. Jpn J Clin Oncol 2009;39(7):425-430

Appendix

Appendix 1 Analysis of Causality Between Drugs and Adverse Events

1) Probably related:

This category applies to AEs that are considered highly likely to be caused by the drugs. 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 while drug-related toxicities persists 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 drugs, 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 meet 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", or "probably related".

	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	+	–	–	–

Appendix 2 ECOG Performance Status Scoring Standard

Score	Performance Status
0	Fully active, able to carry on all pre-disease 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; confined to bed for no more than 50% of waking hours
3	Capable of only limited self care; confined to bed or chair for more than 50% of waking hours
4	Completely disabled; cannot carry on any self care; totally confined to bed or chair
5	Death