Background: Patients receiving pegfilgrastim (Neulasta) for the treatment of neutropenia can experience bone pain following the frequent injections required to achieve effective neutrophil levels. Bone pain is thought to be caused by the rapid expansion of bone marrow hematopoietic cells and accompanying secretion of certain cytokines. ANF-Rho (ANF) is a novel modified granulocyte colony stimulating factor that was found in preclinical animal studies to be approximately 4 fold more potent than pegfilgrastim. A Phase I study was conducted to determine its safety profile and the potential to reduce the occurrence of side effects such as bone pain as well as its dose-response relationship in healthy adults.

Methods: This was a first-in-human, double-blind, randomized, placebo-controlled, single ascending dose Phase I study in 76 healthy subjects. Subjects received a single subcutaneous (sc) dose of ANF over a range of 6 doses (5-50 µg/kg), placebo (saline) or the recommended (sc) dose of ANF over a range of 6 doses (50 µg/kg). The primary outcome measure was safety and tolerability. Secondary outcomes included pharmacokinetics (PK) and pharmacodynamic (PD) effect on absolute neutrophil count (ANC) and CD34+ cell levels. The Bond and Lader Visual Analog Scale (VAS) was used to assess the severity of bone pain.

Results: ANF was well to moderately-well tolerated up to a dose level of 50 µg/kg and appeared to be better tolerated than pegfilgrastim. There were no deaths or withdrawals due to adverse events (AEs) during this study. A total of 354 AEs were reported by 71 (93%) of the subjects. The majority of the AEs were of mild intensity (87%), and 13% of the AEs were of moderate intensity. The most frequently reported were musculoskeletal and connective tissue disorders. Mean bone pain scores were lower in the 5 to 30 µg/kg ANF groups compared to the pegfilgrastim group and were similar for the 50 µg/kg ANF and pegfilgrastim group. There were no clinically significant findings for ANF with respect to clinical laboratory, vital signs, ECG, Holter monitoring, physical examination, and local tolerability. One patient was positive for a low level of anti-drug antibody to ANF. The T1/2 of ANF ranged between 38.5 and 51 hours (hr) and is longer compared to pegfilgrastim (28hr).

Clinical Pharmacodynamics: Blood plasma samples were collected at indicated time points and drug levels were determined by GC/MS (tops) and absolute neutrophil counts and CD34+ cells were determined by flow cytometry (bottoms). Mean (± standard error) drug and ANC values for each cohort were expressed as a function of time post administration (left). Time to peak and area under the curve was calculated for both drug and ANC (right) and data are expressed as mean ± 95% confidence intervals. Asterisks indicated significant differences between indicated groups by ANOVA and Dunnet’s multiple comparison test (p<0.05). PK vs PD. Mean (standard error) drug and ANC levels for 50 µg/kg ANF-Rho and 150µg/kg Neulasta are expressed as a function of time post administration (1st panel, right). Mean PD-ANC-AUC are shown as a function of PK-AUC for each drug (2nd panel, right).