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 clinical dose of pegfilgrastim (100 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 numbers. The Bond and Lader Visual Analogue 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 t½ of ANF ranged between 38.5 and 51 hours (hr) and is longer compared to pegfilgrastim (28hr). The tmax of ANF is 36 hr and is dose-independent and occurs later than for the pegfilgrastim (16 hr).

ANF-Rho has a Cmax and AUC0–t that are approximately 1.5-fold lower compared to treatment with 100 µg/kg pegfilgrastim. This PD effect appeared to be more sustained in the ANF-Rho dose groups compared to pegfilgrastim.

There was a more gradual and prolonged increase of ANC and CD34+ cell by ANF as compared to pegfilgrastim. A maximum mean ANC (8.6 ± 109/L) and CD34+ cell (% 45±10% of 50 µg/kg) was reached on Day 6 and Day 10 as compared to Day 4 for pegfilgrastim (21±50%) (Fig A, B). A maximum number of CD34+ cells (10.7±4 µg/kg) and 71.4 (50 µg/kg) cells/L was reached on Day 7 as compared to Day 5 for pegfilgrastim (66.98 cells/L). The 20 µg/kg ANF dose showed a PD effect (ANC and CD34+) that was comparable to treatment with 100 µg/kg of pegfilgrastim, whereas Cmax and AUC values were 4.5±1 and 2-fold, respectively, lower for the 20 µg/kg ANF dose compared to pegfilgrastim. This PD effect appeared to be more sustained in the ANF-Rho dose groups compared to pegfilgrastim.

Summary/Conclusion: In healthy volunteers, ANF was administered at a dose of 50µg/kg without significant adverse effects. ANF appeared to be better (5 to 30 µg/kg) or equally well (50 µg/kg) tolerated and had lower mean bone pain scores as compared to pegfilgrastim. ANF achieved CD34+ and ANC numbers at significantly lower doses and had a significantly longer circulating half-life than pegfilgrastim. These results suggest that ANF can be provided less frequently at a lower dose and with fewer side effects. Phase 2 trials of ANF are planned to begin shortly in patients.