ANF-Rho: A dose-ranging Phase I study to determine safety, pharmacokinetics and pharmacodynamics in Healthy Volunteers

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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 1 study in 76 healthy subjects. Subjects received a single subcutaneous (sc) dose of ANF over a range of 6 doses (5-50 ug/kg), placebo (saline) or the recommended clinical dose of pegfilgrastim (100µg/kg). The primary outcome measure was safety and tolerability. Secondary outcomes included pharmacokinetics (PK) and pharmacodynamic (PD) effect on absolute neutrophil count (ANC) and number of CD34+ progenitor cells. The Bond and Lader Visual Analogue Scale (VAS) was used to assess the severity of bone pain.

Cohort Summary			
Test Article	Dosage (µg per kg)	Concentraion (mg per ml)	Number of Subjects
Placebo	-	2	13
ANF-Rho	5	2	3
	10	2	8
	15	2	8
	20	2	8
	20HC	5	8
	30	5	8
	50	5	8
Neulasta	100	10	8

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The Conflict of Interest disclosure forms for above authors have been satisfied.

Safety

Cohort	Placebo	ANF-Rho 5 µg/kg	ANF-Rho 10 µg/kg	ANF-Rho 15 µg/kg	ANF-Rho 20LC µg/kg	ANF-Rho 20 HC µg/kg	ANF-Rho 30 µg/kg	ANF-Rho 50 µg/kg	Neulasta* 100 µg/kg	Total
No. of patients enrolled	13	3	8	8	8	8	8	8	12	76
No. of adverse events	E (N) %	E (N) %	E (N) %	E (N) %	E (N) %	E (N) %	E (N) %	E (N) %	E (N) %	E (N) %
ADVERSE EVENTS (total subjects)	20 (11) 85 %	10 (3) 100 %	15 (6) 75 %	44 (8) 100 %	13 (7) 88 %	27 (6) 75 %	31 (7) 88 %	50 (8) 100 %	75 (12) 100 %	285 (68) 89 %
Back pain	2 (2) 15 %	2 (2) 67 %	2 (2) 25 %	12 (7) 88 %	2 (1) 13 %	6 (4) 50 %	5 (4) 50 %	7 (7) 88 %	22 (12) 100 %	60 (41) 54 %
Headache	4 (4) 31 %		4 (4) 50 %	5 (4) 50 %	1 (1) 13 %	8 (4) 50 %	5 (3) 38 %	5 (4) 50 %	17 (9) 75 %	49 (33) 43 %
Injection site pain	1 (1) 8 %	2 (2) 67 %	1 (1) 13 %	4 (4) 50 %	7 (7) 88%	2 (2) 25 %	2 (2) 25 %	3 (3) 38 %	4 (4) 33 %	26 (26) 34 %
Pain in extremity	1 (1) 8 %	1 (1) 33 %	2 (2) 25 %	5 (3) 38 %		2 (1) 13 %	4 (4) 50 %	6 (3) 38 %	6 (6) 50 %	27 (21) 28 %
Arthralgia		3 (2) 67 %		5 (3) 38 %		1 (1) 13 %	5 (3) 38 %	9 (5) 63 %	2 (2) 17 %	25 (16) 21 %
Myalgia	3 (1) 8 %		2 (2) 25 %	5 (4) 50 %				4 (3) 38 %	6 (2) 17 %	20 (12) 16 %
Bone pain				2 (2) 25 %	1 (1) 13 %	1 (1) 13 %	3 (3) 38 %	2 (2) 25 %	2 (2) 17 %	11 (11) 14 %
Musculoskeletal pain	1 (1) 8%		1 (1) 13 %			1 (1) 13 %	2 (1) 13 %	2 (2) 25 %	4 (3) 25 %	11 (9) 12 %

e: number of events n: number of patients experiencing event %: No of patients experiencing events/total enrolled patients

Drug related Treatment Emergent Adverse Events (TEAE)

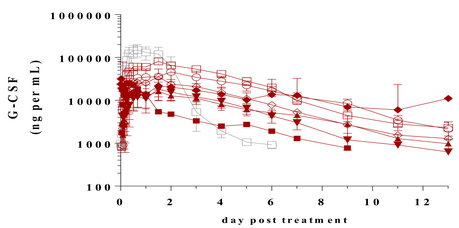
Sixty-eight of the 76 (89%) subjects reported at least one TEAE that was considered possibly or probably related to the study drug by the PI. In total, 285 of the 354 TEAEs (81%) were considered possibly or probably to be related to the study drug (ANF-Rho, Neulasta® or placebo) by the PI. The profile of the most frequently reported related TEAEs was similar to the TEAE profile overall. The TEAEs considered to be related to the study drug by the PI and that were reported in at least 10% of the subjects:

Several TEAEs were treated with concomitant medication, as allowed by protocol. Paracetamol was sufficient for the treatment of back pain or other musculoskeletal pain, except for one subject who was treated with ibuprofen to treat shoulder pain. Concomitant medication was mostly given to subjects in the 50 µg/kg ANF-Rho group and Neulasta® group.

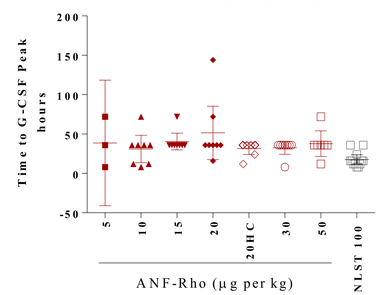
Overall, treatment with one single sc dose of up to 50 µg/kg ANF Rho was well to moderately well tolerated. When compared to Neulasta®, ANF-Rho appeared to be equally well (50 µg/kg dose group) or better (5 to 30 µg/kg dose groups) tolerated than the reference Neulasta®.

Pharmacokinetics

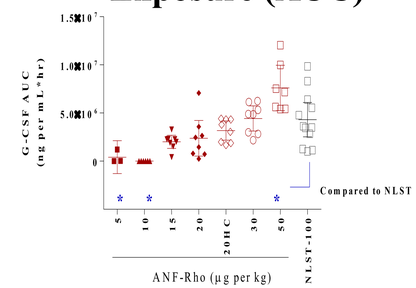
Response vs. Time



Time to Peak

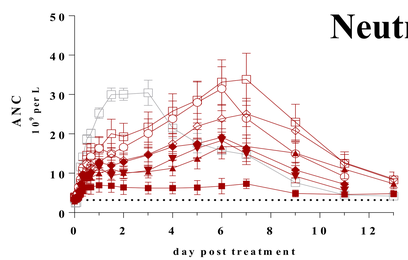


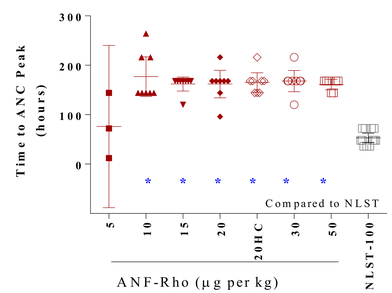
Exposure (AUC)

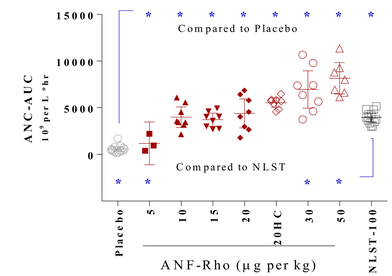


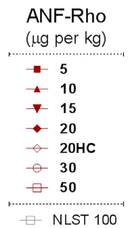
Pharmacodynamics

Neutrophils

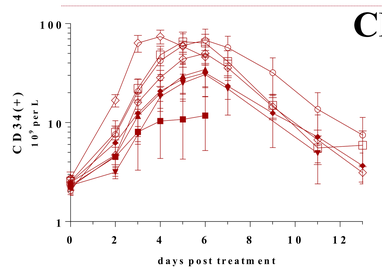


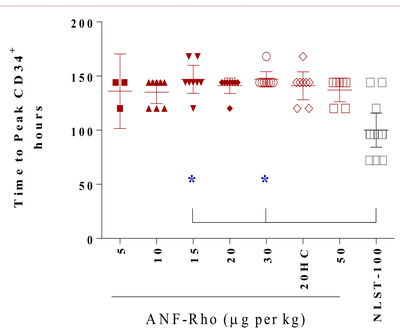


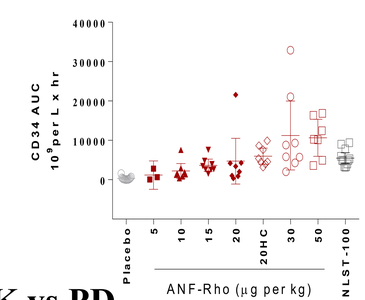




CD34+



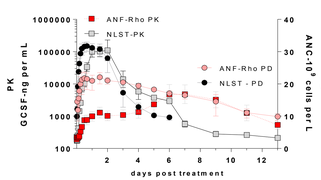


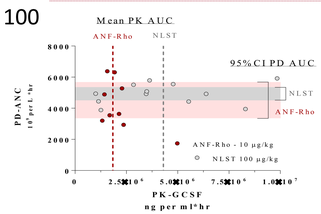


Pharmacokinetics & Pharmacodynamcis. Blood plasma samples were collected at indicated time points and drug levels were determined by G-CSF ELISA (top) and absolute neutrophil counts and CD34+ cells were determined by flow cytometry (bottom). Mean (± standard error) drug and ANC values for each cohort 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 and 95% confidence intervals. Asterisks indicated significant differences between indicated groups by ANOVA and Dunnet's multiple comparison tests ($p<0.05$). PK vs PD. Mean (±standard error) GCSF and ANC levels for 10µg per kg ANF-Rho and 100µg per kg NLST 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)

PK vs PD

ANF-Rho 10 vs. NLST 100





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). 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 and AUC0-inf values that increase with dose without a dose-linear relationship, a Cmax approximately 1.8-fold lower for the 50 µg/kg dose and a mean AUC0-t and AUC0-inf that are approximately 1.5-fold higher compared to treatment with 100µg/kg 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 x109/L (5 µg/kg) and 45x109/L (50 µg/kg) was reached between Day 6 and Day 10 as compared to Day 4 for pegfilgrastim (21.5x109/L) (Fig A, B). A maximum number of CD34+ cells (10.74 (5 µg/kg) and 71.4 (50 µg/kg) cells/µL) was reached on Day 7 as compared to Day 5 for pegfilgrastim (66.98 cells/µL). The 20 µg/kg ANF dose showed a PD effect (ANC and CD34+) that was comparable to treatment with 100µg/kg of pegfilgrastim, whereas Cmax and AUC values were 4.3-fold and 1.7-fold, respectively, lower for the 20 µg/kg ANF dose compared to the 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.