PRELIMINARY RESULTS FROM A LONG-TERM REPEAT DOSE TOXICITY AND TOXICOKINETIC STUDY OF ANF-RHO™, A NOVEL ANTI-NEUTROPENIC FACTOR

Heman Misra, PhD, Judith A Newmark, PhD. (1Prolong Pharmaceuticals, South Plainfield, NJ, 2Toxikon, Bedford, MA)

Methods: The study design used 288 rats, divided into 5 dosage groups: control, 100, 300, 1000 (high) and 1000 (positive) μg/kg. A total of 58 monkeys were also divided into 5 dosage groups: control, 75, 250, 750 (high dose) and 750 (positive) μg/kg of ANF-RHO™. Doses were administered by weekly subcutaneous injections on Day 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 81, 82 and 84 at a dose volume of 5 mL/kg. Genotoxicity assessments were evaluated using Salmonella typhimurium and Escherichia coli reverse mutation assay, rodent blood micronucleus assay, and chromosomal aberration assay. Toxicology assessment included clinical observations, body weight change, food consumption, ophthalmic examination, function observational battery (motor activity, behavioral changes, coordination and sensory/motor reflex response), organ weight, bioanalytical and toxicokinetic analysis, immunogenicity, gross necropsy, and histopathology.

Results: No observed clinical signs seemed to be related to ANF-RHO™ administration. There were no related effects in body weight changes or food consumption. Observed ophthalmic effects were considered procedural related due to low incidence. No biologically meaningful findings were noted during the function observational battery assessment. Preliminary analysis showed a dose related increase in spleen weight in rats and a dose dependent decrease in kidney weight in primates. Genotoxicity studies found no signs of mutagenicity, clastogenicity or cytotoxicity.

Background: ANF-RHO™ is a novel polyethylene glycol-modified granulocyte colony stimulating factor that has biophysical and biological properties that produce a prolonged pharmacokinetic and pharmacodynamic profile as compared to pegfilgrastim (Neulasta®). As such, it has potential applications in chemotherapy induced neutropenia and chronic idiopathic neutropenia. These disorders require prolonged administration of G-CSF agents to treat neutropenia. Therefore, long term toxicity, genotoxicity and juvenile studies were conducted with ANF-RHO™.

Aim: A 13-week study was conducted in Sprague Dawley rats and cynomolgus primates to assess various safety and pharmacokinetics of ANF-RHO™ as compared to Neulasta® (pegfilgrastim).

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Dosing Design for 3 Month Repeat Dose in Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Number of Animals / Sex</th>
<th>Dose µg/kg per Injection</th>
<th>Day 93 Main Concentration (µg/mL)</th>
<th>Day 120 Main Concentration (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7M / 7F</td>
<td>0</td>
<td>Control</td>
<td>Control</td>
</tr>
<tr>
<td>Low Dose</td>
<td>4M / 4F</td>
<td>2</td>
<td>0.2 µg/mL</td>
<td>0.2 µg/mL</td>
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<tr>
<td>Mid Dose</td>
<td>4M / 4F</td>
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<td>1.0 µg/mL</td>
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<td>4M / 4F</td>
<td>10</td>
<td>10.0 µg/mL</td>
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Results of 3 Month Repeat Dose in Rats

Toxicokinetic Analysis for 3 Month Repeat Dose in Rats

Dosing Design for 3 Month Repeat Dose in NHP

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Results of 3 Month Repeat Dose in NHP

Toxicokinetic Analysis for 3 Month Repeat Dose in NHP

Immunogenicity Analysis for 3 Month Repeat Dose in NHP

Genetic Toxicology: Ames Test

Objective
- Assess potential mutagenic effect of ANF-RHO™
- OECD 471
- Bacterial 4 strains of Escherichia coli and 1 strain of Salmonella typhimurium.
- With and without metabolic activator
- 2 – 0.1 mg per plate

Results
- No biologically and statistically significant increase compared to controls
- Not mutagenic

Genetic Toxicology: Chromosomal Aberration

Objective
- Assess potential clastogenic and mutagenic effect of ANF-RHO™ in mammalian cells
- OECD 473
- Human Peripheral Blood Lymphocytes
- With and without metabolic activator
- 2.2 – 0.2 mg/mL exposure

Results
- No biologically and statistically significant increase compared to controls
- Not clastogenic

Genetic Toxicology: Micronucleus

Objective
- Assess potential clastogenic effect of ANF-RHO™ in vivo
- OECD 474
- Mouse: 5 Males and 5 Females per dose and time group
- 3 groups - 260 mg/kg
- Blood collected 36 and 48 hours after last administration
- Flow cytometry analysis of 20,000 reticulocytes

Results
- No dose response at biologically insignificant increase of micronuclei frequency compared to controls
- Not clastogenic

Conclusion
ANF-RHO™ is a granulocyte-stimulating factor that has a unique pharmacokinetic and pharmacodynamic profile as compared to pegfilgrastim. The current study was performed to provide support in indications which may require long-term administration of ANF-RHO™. The results from this preliminary toxicology studies are unremarkable and consistent with those of an earlier 28-day study. Results from the 3-7 day neutropenia dosing model found that the blood pharmacodynamics parameters of ANF-RHO™ are significantly superior to pegfilgrastim. Both PK and PD data demonstrate relatively predictable systemic exposure and activity following SC or IV dose levels in both rat and primate. The unique PK/PD studies in animals and in healthy volunteers suggest that ANF-RHO™ may be dosed at a significantly lower level as compared to pegfilgrastim, thereby potentially reducing side effects such as bone pain while mitigating severe neutropenia that follows chemotherapy. These long-term 13-week toxicology studies provided evidence of safety sufficient to support advancement of ANF-RHO™ into Phase II clinical studies in chemotherapy-induced neutropenia and chronic idiopathic neutropenia in Europe, USA, and India.