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A multiple-dose toxicity study of tanezumab in cynomolgus monkeys

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ABSTRACT

Nerve growth factor (NGF) is an important mediator of pain and hyperalgesia and has become a target of novel analgesic therapeutics. Tanezumab is a humanized IgG_2 antibody that binds NGF with high affinity and specificity. In a study to assess the toxicity and pharmacokinetic properties of tanezumab in adult, male and female, cynomolgus monkeys following weekly intravenous administration of 1, 10, or 30 mg/kg for up to 26 weeks (followed by an 8-week recovery period), tanezumab was well tolerated with no macroscopic or microscopic effects on those brain, spinal cord, nerve, or ganglia sections evaluated. One fifth of tanezumab-treated monkeys developed an antibody response to tanezumab that prevented maintenance of tanezumab exposure between dosing. In the antibody-negative animals, accumulation of tanezumab was observed; steady state was achieved approximately 8 weeks after the first dose of study drug, and exposure to tanezumab was approximately dose proportional with no observed difference between male and female animals. One monkey died during the study; this monkey had findings suggestive of hypersensitivity reaction. The favorable toxicity and pharmacokinetic profile of tanezumab seen in this study supports its further evaluation for the treatment of pain in clinical practice.

1. Introduction

Nerve growth factor (NGF) has been known to play a role in the development of the nervous system since its discovery some 50 years ago (Cohen, 1960; Levi-Montalcini and Booker, 1960). However, since the early 1990s various roles for NGF in mature adults have also been proposed, including a role in the modulation of neuronal structure and function. It has, moreover, been shown to be an important mediator of pain and hyperalgesia (Della Seta et al., 1994; Lewin et al., 1993; McMahon et al., 1995; Woolf et al., 1994). These findings suggested that NGF may be a viable target of novel analgesic therapeutics. Consequently, there have been several attempts to develop new pain treatments based on NGF antagonism (Hefti et al., 2006; Watson et al., 2008).

Tanezumab is a humanized IgG_2 antibody that has been shown to bind NGF with high affinity and specificity and block binding to both of its known receptors, trkA and p75 (Abdiche et al., 2008). In animal models of arthritis, bone cancer, bone fracture, complex regional pain syndrome, and post-operative pain, tanezumab and its murine precursor, muMab911, have been associated with improvements in pain-related behaviors, with no effect on normal pain sensitivity in naïve animals (Koewler et al., 2007; Sabsovich et al., 2008; Sevcik et al., 2005; Shelton et al., 2008, 2005). Moreover, the efficacy of tanezumab in reducing pain-related behaviors in some animal models was comparable to, or greater than that achieved with morphine sulfate and superior to that achieved with non-steroidal anti-inflammatory drugs (Halvorson et al., 2005; Jimenez-Andrade et al., 2007; Koewler et al., 2007; Sevcik et al., 2005).

This paper reports the design and results of the most extensive tanezumab non-clinical toxicology study conducted to date – a 26-week multiple dose study with an 8-week recovery period, conducted in cynomolgus monkeys. This study is part of the preclinical safety program conducted before a clinical trial program can be initiated to evaluate the use of tanezumab in humans. Cynomolgus monkeys were chosen as the test species for the program because NGF in this species has identical sequence homology to that in humans and in both species tanezumab binds with high affinity to a common epitope as determined by Plasmon Resonance Binding. Administration to monkeys also presents the opportunity to evaluate possible safety considerations following repeated chronic administration of tanezumab, while minimizing the chances of a universal anti-drug antibody (ADA) response. Tanezumab dose selection (1, 10, and 30 mg/kg) was based on tanezumab exposures observed in a previous 4-week multiple-dose study in the same species, at the same doses, in which the highest dose employed resulted in plasma exposure to drug that was at least 100 times

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greater than the plasma exposure achieved with the dose anticipated for use in clinical practice. Tanezumab was administered by the intravenous (IV) route in the current study as this is an intended route of administration in humans. The objectives of this study were to assess the potential toxicity of tanezumab and to evaluate its pharmacokinetic (PK) properties in adult monkeys following multiple-dose, weekly, IV administration of 1, 10, and 30 mg/kg for up to 26 weeks.

2. Materials and methods

2.1. Test and control articles

Tanezumab and control (placebo aqueous solution of 10 mM histidine buffer, 275 mM sucrose and 0.01% tween-20 at pH 6.0) were manufactured at Lonza (Slough, UK), put into vials by Alliance Medical Products and stored at −15 °C and 2–8 °C, respectively. Tanezumab solutions were thawed at 2–8 °C, and diluted as required on the day of dosing. Final dosing solutions were tested for stability and checked for concentration using a validated UV method. The analytical results verified that the solutions had been prepared properly and were stable under the conditions of use.

2.2. Test animals and husbandry

Cynomolgus monkeys were supplied by Primate Products (Miami, FL, USA) and acclimatized to laboratory conditions for at least 30 days prior to receiving the first dose of study medication. Monkeys were young adults (>2 years old); males ranged in weight from 2.0 to 2.9 kg and females from 2.0 to 2.5 kg at the first dose. The monkeys were kept in steel cages (one animal per cage) with the room temperature maintained at 17.8–28.9 °C and food and water provided *ad libitum*.

This study was conducted at Gene Logic Laboratories, Inc. (Gaithersburg, MD, USA) in compliance with current US FDA Good Laboratory Practice Regulations for Non-Clinical Laboratory Studies (21 CFR Part 58); the Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (as revised in 1997; ENV/MC/CHEM (98) 17); and the Japanese Ministry of Health and Welfare (MHLW) Good Laboratory Practice Standards for Safety Studies on Drugs (MHLW Ordinance Number 21, March 26, 1997). The study protocol complied with the USDA Animal Welfare Act; the Public Health Service Policy on Humane Care and Use of Laboratory Animals; and the US Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals.

2.3. Study design

Monkeys were eligible for randomization based on body weight and physical examination. Male and female monkeys were separately randomized to receive weekly doses of placebo or tanezumab 1 mg/kg, 10 mg/kg, or 30 mg/kg using computer-generated random numbers such that there were four male and four female animals in each treatment group (Fig. 1). An additional two males and two females, assigned as 'recovery' animals, were randomized to the control and tanezumab 30 mg/kg per week groups. Surviving ADA-negative animals completed an 8-week treatment free period following the 26-week treatment period. At randomization, there was no statistically significant difference between treatment groups in mean body weight. Treatment was administered weekly by IV bolus injection over 2 min. in the saphenous vein (alternating between left and right) for the intended period of 26 weeks. Following their participation in the study, animals were euthanized by vascular perfusion with formaldehyde solution under deep anesthesia with Nembutal®. This method ensured the best structural preservation of nervous tissue.

2.4. Observations

Cageside, clinical, and ophthalmological observations/measurements were conducted according to the schedule shown in Table 1. Cageside observations included observation for mortality, moribundity, general health, and signs of toxicity. Clinical observations included evaluation of skin and fur characteristics, eye and mucous membranes, respiratory, circulatory, autonomic, and central nervous systems, and somatomotor and behavior patterns. Ophthalmological examinations were performed by indirect ophthalmoscopy following administration of 1% tropicamide mydriasis. Blood pressure, electrocardiogram (ECG), body temperature, and respiratory rate were collected while animals were anesthetized with Telazol® (4 mg/kg).

2.5. Anti-tanezumab and pharmacokinetic analyses

Blood samples (~0.5 ml) for the determination of ADA concentrations and PK analysis were collected from the femoral vein into tubes containing lithium heparin according to the schedule in Table 1. Following the decision to withdraw animals early from treatment due to suspected antibody formation and risk of hypersensitivity to tanezumab the study protocol was amended to allow blood collection on Day 155 for all treatment groups (with Day 155 replacing Day 176 in those animals being discontinued). Samples were centrifuged at ~3000 rpm at 4 °C for 10 min., then transferred to microcentrifuge tubes and stored at −75 ± 10 °C before transport to the analytical facility where they were stored at −70 ± 10 °C pending analysis.

Plasma levels of tanezumab and of anti-tanezumab antibodies were evaluated using validated Enzyme-Linked Immuno Sorbent Assays (ELISA). For the PK assay, recombinant human NGF was immobilized onto microtiter plates. The validated bioanalytical assay range was 2.88–92.1 ng/mL. The minimum dilution was 1:100 (using a dilution buffer). The minimum quantifiable concentration of tanezumab (given the ±20.49% acceptance criterion) was 229 ng/mL. Dilution integrity was established using 1% monkey plasma with a dilution linearity for total sample dilutions of up to 200,000 fold. Long term plasma tanezumab stability has been validated for over 294 days at −70 °C. The intra- and inter-assay precision of the validated assay in the sample matrix was 6.26–11.2% and 9.57–19.2%, respectively.

In the ADA assay positive control/reference standard (goat anti-human IgG (H + L) and test samples were incubated with tanezumab, which had been immobilized on an ELISA plate. After incubation, unbound material was washed away and cynomolgus anti-tanezumab antibodies detected using biotinylated tanezumab followed by HRP-streptavidin conjugate, and visualized with 3,3′,5,5′-tetramethylbenzidine. Results were reported as positive or negative for antibodies to tanezumab.

As with most immunoassays used for immunogenicity testing, circulating drug can interfere with the assay, potentially producing false negative results. Tanezumab concentrations of 100 ng/ml resulted in 90% inhibition of the positive control when tested at a 1:6400 dilution. The sensitivity of the assay was not determined at the time of validation since a drug specific positive control was not available. A confirmatory assay to demonstrate the specificity of the responses in samples with a positive response was not performed. The intra- and inter-assay precision of the validated assay over the range of standard reference curve dilutions was 0–17.2% and 3.82–7.51%, respectively. ADA and PK analyses were conducted at Prevalere Life Sciences, Inc. (Whitesboro, NY, USA).