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Characterization and re-evaluation of experimental pain models in healthy subjects

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Chapter 3

DEMONSTRATION OF AN ANTI-HYPERALGESIC EFFECT OF A NOVEL PAN TRK INHIBITOR PF-06273340 IN A BATTERY OF HUMAN EVOKED PAIN MODELS

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ABSTRACT

Inhibitors of nerve growth factor (NGF) reduce pain in several chronic pain indications. NGF signals through tyrosine kinase receptors of the tropomyosin-related kinase (Trk) family and the unrelated p75 receptor. PF-06273340 is a small molecule inhibitor of Trks A, B and C that reduces pain in nonclinical models and this study aimed to investigate the pharmacodynamics of this first in class molecule in humans.

A randomized, double blind, single dose, placebo and active-controlled 5 period cross-over study was conducted in healthy human subjects (NCT02260947). Subjects received 5 treatments: PF-06273340 50 mg, PF-06273340 400 mg, pregabalin 300 mg, ibuprofen 600 mg and placebo. The 5 primary endpoints were the pain detection threshold for the thermal pain tests (normal and UVB skin) and the pain tolerance threshold for the cold pressor, electrical stair and pressure pain tests. The trial had pre-defined decision rules based on 95% confidence that the PF-06273340 effect was better than placebo.

20 subjects entered the study, with 18 completing all 5 periods. The high dose of PF-06273340 met the decision rules on the UVB skin thermal pain endpoint (LSmeans versus placebo: 1.13, 95% confidence interval: 0.64-1.61), but not on the other 4 primary endpoints. The low dose did not meet the decision criteria for any of the 5 primary endpoints. Pregabalin (cold pressor and electrical stair) and ibuprofen (UVB thermal pain) showed significant analgesic effects on expected endpoints.

This study demonstrated for the first time translation of nonclinical effects into man in an inflammatory pain analgesic pharmacodynamic endpoint using a pan-Trk inhibitor.

INTRODUCTION

Nerve growth factor (NGF) is a key mediator of chronic pain. Administration of NGF to animals or to human subjects causes pain,¹ and studies with anti-NGF scavenging monoclonal antibodies such as tanezumab have demonstrated efficacy in Phase 3 trials in several chronic pain indications.^{2,3} NGF is a member of the neurotrophin family that signal through both tyrosine kinase receptors of the tropomyosin-related kinase (Trk) family and the unrelated p75 receptor. The neurotrophins comprise NGF which signals preferentially through TrkA, brain-derived neurotrophic factor (BDNF) and neurotrophin 4 (NT4) which signal through TrkB, and neurotrophin 3 (NT3) which signals through TrkC. The neurotrophins are equipotent at the p75 receptor. NGF signaling through TrkA is known to induce both acute and chronic regulation of pain signaling, through phosphorylation dependent regulation of ion channels involved in pain transmission and upregulation of pain related genes respectively.⁴ BDNF was also found to be implicated in nonclinical pain signaling and was found to be upregulated in clinical pain states in human subjects.⁵

PF-06273340 is a peripherally restricted small molecule inhibitor of Trks A, B and C, where the structural formula has been published previously.⁶ It is equipotent at the three Trk receptors, but is otherwise broadly selective. PF-06273340 and other molecules in this class reverse chronic pain in nonclinical models where there has been some sensitization such as UVB sensitization to the skin⁶ or carrageenan irritation of the joint.⁷ To date there are no data on whether these analgesic effects of small molecule pan Trk inhibitors translate to human subjects.

In the current study the analgesic effects of PF-06273340 were assessed using a battery of human evoked pain models. These models have been shown to provide robust evidence of analgesia in healthy human subjects using a number of positive controls assessed against different pain stimuli and endpoints.^{8,9} By assessing PF-06273340 using this methodology we intended to establish whether the nonclinical data demonstrating efficacy in the UVB sensitization model would translate to human subjects. The inclusion of other pain models in the study provided a comparison with non-sensitised pain states. Furthermore we would be able to provide the first demonstration of analgesic efficacy for this novel class of compounds in a small, easy to recruit trial prior to investing in larger patient studies.

METHODS

Subjects and study design

The study was a double blind, double dummy, single dose, randomized, placebo-controlled, 5-period cross-over study (NCT02260947). In this study PF-06273340 was the drug under investigation and ibuprofen and pregabalin were used as positive controls. The study was conducted at a single site at the Centre for Human Drug Research in Leiden, The Netherlands. The study was approved by the Medical Ethics Committee of Stichting Beoordeling Ethiek Biomedisch Onderzoek (Assen, The Netherlands). The study was conducted according to the Dutch Act on Medical Research Involving Human Subjects (WMO) and in compliance with all International Conference on Harmonization Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. Each subject provided written informed consent before any screening procedures were performed.

Approximately 20 healthy male subjects, between 18 to 55 years of age, were to attend the clinic on 7 separate occasions (Screening, Periods 1-5 and follow-up) to examine the effects of PF-06273340 on evoked pain endpoints. Periods 1-5 were spaced apart by at least 7 days, which based on the half-lives of the single doses of treatments would give sufficient time to allow for the wash-out of pharmacokinetic (PK) and potential pharmacodynamic (PD) effects.

Study drugs

The study included placebo, ibuprofen and pregabalin (positive controls) and 2 dose levels of PF-06273340. Subjects were randomised to one of 10 sequences that consisted of two Williams 5x5 design that were balanced for first-order carry-over. At each investigational period, subjects received a total of 4 tablets and 1 capsule: 2 tablets of 200 mg PF-06273340/placebo, 1 tablet of 50 mg PF-06273340/placebo, 1 tablet 600 mg ibuprofen/placebo and 1 capsule 300 mg pregabalin/placebo. The doses of PF-06273340 selected for this study were 50 mg and 400 mg given as single doses. These doses were justified based on margins to toxicology findings and on clinical toleration and safety data from Phase 1 single and multiple ascending dose studies (<https://clinicaltrials.gov/ct2/results?term=PF-06273340>) in healthy young and elderly subjects. The top dose of 400 mg PF-06273340 was close to the maximum dose given previously to human subjects, whereas the lower dose of 50 mg PF-06372865 allowed examination of the bottom end of the predicted pharmacologically active range.

Doses of 600 mg ibuprofen and 300 mg pregabalin had been used as positive controls in previous human evoked pain model studies.^{8,9} These doses were well tolerated and within the labeled dose range for ibuprofen and pregabalin in the European Union (EU).

Pharmacokinetic assessments

During all study periods, blood samples (3 mL) to provide a minimum of 1.5 mL plasma for PK analysis PF-06273340, pregabalin and ibuprofen were collected at pre-dose and 0.5, 1, 2, 3, 4, 5, 6, 8 and 10 hours after study drug administration for PK analysis of PF-06273340, pregabalin and ibuprofen. Plasma PF-06273340 pharmacokinetic parameters: maximum observed plasma concentration (C_{max}), area under the plasma concentration-time profile from time 0 to the time of last quantifiable concentration (AUC_{last}), time for C_{max} (T_{max}) were calculated for each subject using non compartmental analysis of plasma concentration-time data.

Pharmacodynamic assessments

Pain detection and tolerance thresholds were measured using a battery of human pain models that assess a range of modalities of pain using previously described methodology.^{8,9} Briefly, the pain models included thermode, electrical stimulation, mechanical pain and cold pressor, which were all performed sequentially at pre-dose (twice) and 0.5, 1, 2, 3, 4, 6, 8 and 10 hours after study drug administration in each period. Thermal (heat) pain was determined on normal skin and on UVB exposed skin. Pain intensity was measured continuously during each test using an electronic visual analogue scale (eVAS) ranging from 0 (no pain) to 100 (most intense pain tolerable). The pain detection threshold (PDT) and pain tolerance threshold (PTT) were of primary interest (see Supplementary data for more details).

Ibuprofen and pregabalin were included as active controls as based on previous studies;^{8,9} ibuprofen had shown effects on the UVB Heat PDT endpoint and pregabalin had shown effects on the Cold Pressor PTT, Pressure Pain PTT, Electrical Stair PTT (pre-Cold pressor) and Normal Heat PDT endpoints.

Statistical Analysis

A mixed effects model was fitted to each endpoint, using data collected during the first six hours post treatment. Absolute values were analysed for PDT endpoints and $\log_e$ transformed values for PTT endpoints as the latter had skewed distributions

in previous studies.^{8,9} The fixed effects included in the model were baseline, period, time, treatment and treatment by time interaction, with baseline as covariate. Subject was fitted as a random effect. Baseline was included as two separate variables, the average baseline for the subject, and the deviation of each period baseline from the average baseline for each subject.¹⁰ The Kenward-Roger approximation was used for estimating degrees of freedom for the model parameters. The primary analysis included all subjects randomised into the study.

The LSMeans together with 90% confidence intervals were obtained for each treatment averaged across time points that cover the peak exposure of each treatment. Based on the known human pharmacokinetics the average across the first 4 hours was obtained from the mixed effects model for PF-06273340 and Ibuprofen whereas the average across the first 6 hours was obtained for pregabalin. Both the average over the first 4 and 6 hours were obtained for placebo. Differences between treatments and placebo were therefore made using the appropriate average (*i.e.*, ibuprofen was compared to the placebo 4 hour average, whereas pregabalin was compared to the placebo 6 hour average). Differences to placebo are presented as absolute differences for PDT endpoints and ratios for PTT endpoints together with corresponding 90% confidence intervals.

As a sensitivity analysis to the primary analysis, a mixed effects model was fitted for the maximum (over 4 or 6 hours post treatment, where appropriate) change from baseline for each primary endpoint. The fixed effects included in the model were baseline, period and treatment. Baseline was similarly included as 2 separate variables. Subject was fitted as a random effect. Additional sensitivity analyses were conducted that applied the primary analysis models to only subjects who completed all 5 treatment periods or applied the models to all subjects but included all time points (*i.e.*, up to and including the 10 hour measurement).

Sample Size

Decision rules were pre-specified to quantify what was required in the primary objective of the study. The criteria were based on a Bayesian interpretation of the results assuming a non-informative prior. The criterion used for each endpoint was: At least 95% confident that either dose of PF-06273340 effect was greater than placebo. This is equivalent to a one-sided test for statistical significance using an alpha of 0.05. No adjustment was made for multiplicity as this was an early phase clinical study designed to explore the pharmacodynamics of PF-06273340 and as such no stringent requirement to control the type 1 error rate was required for internal decision making.

The sample size was based on the mean effect over the first 4 hours after dosing (*i.e.*, average of 0.5, 1, 2, 3 and 4 hour time points) for the 5 primary endpoints: Cold Pressor PTT; Pressure Pain PTT; Electrical Stair PTT; Normal Heat PDT; and UVB Heat PDT. The primary comparison was of either dose of PF-06273340 against placebo. A conservative estimate of within-subject standard deviation was derived from two previous methodology studies.^{8,9} yielding estimates of 0.25, 0.21, 0.16, 1.79 and 1.63 for the Cold Pressor PTT, Pressure Pain PTT, Electrical Stair PTT, Normal Heat PDT, and UVB Heat PDT endpoints, respectively. A sample size of 20 subjects was selected to ensure balance in the design and gave at least 80% power to detect differences of 0.20, 0.17, 0.13, 1.42 and 1.30 for the 5 primary endpoints listed previously.

NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,¹¹ and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16.¹²

RESULTS

Subject disposition

Of the 20 subjects that were randomised, 18 completed the study (Figure 1). Two subjects discontinued from the study: one due to failure to meet inclusion/exclusion criteria (ECG abnormalities) and did not proceed to Period 5 (missed PF-06273340 50 mg); and the other subject due to no longer willing to participate in the study and only received treatments assigned to Periods 1 and 3 (only treated with PF-06273340 50 mg and ibuprofen 600 mg, respectively). All 20 subjects were male, with a mean age (standard deviation) of 26.0 (6.9) and body mass index of 23.7 (2.5) (Table 1). Fifteen subjects reported ethnicity as “White” with the 5 remaining as “Other”. All 20 subjects were dosed with ibuprofen 600 mg, and nineteen subjects were dosed with placebo, PF-06273340 50 mg, PF-06273340 400 mg or pregabalin 300 mg.

Pharmacodynamics

A summary of the results to the primary analyses are presented in Table 2 and Figure 2. PF-06273340 400 mg met the decision criteria for the UVB Heat PDT

endpoint as shown by a statistically significant increase over placebo of 1.13 units (90% CI = 0.64 to 1.61). There were no statistically significant effects of PF-06273340 50 mg relative to placebo on any of the 5 primary endpoints. Ibuprofen showed a statistically significant effect on the UVB Heat PDT endpoint compared to placebo with an increase of 1.39 units (90% CI = 0.91 to 1.87). pregabalin 300 mg had statistically significant effects over placebo on the Cold Pressor PTT (effect versus placebo = 1.22, 90% CI = 1.11 to 1.34) and the Electrical Stair PTT (effect versus placebo = 1.09, 90% CI = 1.01 to 1.19). The time course profiles of the five treatments across the 5 primary endpoints are presented in Figure 3. Sensitivity analyses gave similar results (data not shown).

Safety

Single doses of PF-06273340 400 mg or PF-06273340 50 mg administered to healthy male subjects were generally safe and well tolerated in this study. There were no SAEs or other clinically significant AEs reported.

The most frequently reported all causality treatment related adverse events were dizziness (16 subjects: 4 subjects in PF-06273340 400 mg group, 2 subjects each in PF-06273340 50 mg and placebo group, and 8 subjects in pregabalin group), somnolence (13 subjects: 2 subjects each in PF-06273340 50 mg and ibuprofen group, 1 subjects each in PF-06273340 400 mg and placebo group, and 7 subjects in pregabalin group), and fatigue (11 subjects: 2 subjects each in PF-06273340 400 mg and pregabalin group, 3 subjects each in PF-06273340 50 mg and ibuprofen group, and 1 subject in placebo group). All treatment related adverse events were mild in severity, except 1 subject in PF-06273340 50 mg treatment group had upper abdominal pain, which was moderate in severity and considered treatment-related by the investigator.

Pharmacokinetics (PK)

Median plasma PF-06273340 concentration-time profile is presented in Figure 4 and PK parameters are summarized descriptively in Table 3.

PF-06273340 was rapidly absorbed following oral administration with mean T_{max} of 1 hour for both treatments. C_{max} and AUC_{last} appeared to increase proportionally with doses from 50 mg to 400 mg, and between subject variability in plasma PF-06273340 exposure based on geometric %CV for C_{max} and AUC_{last} ranged from 42% to 57%.

DISCUSSION

This is the first study to test a novel candidate analgesic targeting the NGF pathway using a panel of human evoked pain models. Previous studies had demonstrated that these models provided a reproducible method to assess the effects of analgesic drugs on a battery of evoked pain assessments in healthy human subjects, with consistent results obtained from the ibuprofen and pregabalin positive controls.^{8,9} The current study confirmed previous results showing a significant effect of ibuprofen on the UVB Heat PDT assessment, and of pregabalin on the cold pressor test. pregabalin also demonstrated a modest effect in the electrical stair which achieved statistical significance and had demonstrated an effect at this endpoint in some but not all previous studies.^{8,9} Overall the results of the positive controls ibuprofen and pregabalin confirm the validity of this methodology for detecting reproducible analgesic signals in healthy human subjects.

The 400 mg dose of pan Trk inhibitor PF-06273340 significantly reduced the hyperalgesia seen in the UVB Heat PDT assessment but did not have an effect on any other endpoint. This is similar to the pattern seen with ibuprofen and is in agreement with expected biology of the mechanism. NGF is upregulated in experimental models of inflammation including UVB sensitisation,^{11,12} and anti-NGF monoclonals and Trk inhibitors (including PF-06273340) have shown efficacy in nonclinical models of inflammatory pain such as complete Freund's adjuvant, carrageenan and ultraviolet B radiation.^{6,13} NGF has direct and indirect actions in inflammatory pain (reviewed by Mantyh et al).¹³ Administration of NGF leads to binding at TrkA on immune cells (including mast cells) and subsequent release of inflammatory mediators which contribute to sensitisation of nociceptors. In addition, NGF binding to TrkA on sensory nerve fibres elicits signalling cascades which result in trafficking of nociceptors to the cell surface and their sensitisation by phosphorylation. One of the receptors that contributes to increased signalling in this manner is the heat sensitive ion channel TRPV1 which is likely an important component of the UV induced hyperalgesia in man;¹⁴ inhibition of TRPV1 signalling may be one component of PF-06273340 efficacy in this model. The indirect effects of NGF involve the retrograde transport of NGF/TrkA complexes to the nucleus where the transcription of nociceptors and peptides involved in pain signalling are upregulated. In vitro data have shown that this transport is inhibited by PF-06273340 (Bilsland personal communication), and this may also contribute to the effect on UVB seen in the present study. Although the longer timescale of this process implies it may be more important in chronic pain states.

Given the proven efficacy of anti-NGF monoclonals in nonclinical species and in human clinical studies it is tempting to ascribe the efficacy of PF-06273340 to a blockade of TrkA signalling. However, a role for BDNF (which signals through TrkB) cannot be discounted as it has been shown to play a role in hyperalgesia and pain in some nonclinical systems and has been implicated in some human biology studies in visceral and neuropathic pain states.⁵ The effects of NT3 signalling through TrkC and NT4 signalling through TrkB are not as well defined¹⁵ but published data do not suggest a major role in nociception. One conclusion that can be drawn from the effects of PF-06273340 is that blockade of signalling through p75 (which is spared by PF-06273340 but not by anti-NGF monoclonals) is not required for an analgesic effect in man.

The effect size of the 400 mg dose of PF-06273340 versus placebo on the UVB endpoint was similar to that of ibuprofen, however the lower 50 mg dose of PF-06273340 did not have a significant effect. The median exposure of PF-06273340 at the top dose achieved $\sim 30 \times IC_{50}$ at C_{max} and dropped to $\sim 9 \times IC_{50}$ at the end of the assessment period (4 hours), whereas the lower dose achieved $\sim 4 \times IC_{50}$ at C_{max} and dropped to $\sim 1 \times IC_{50}$ at 4 hours. The differential efficacy of these two doses implies that at least for this endpoint an exposure that achieves a multiple of IC_{50} throughout the assessment period is required for an acute pharmacodynamic effect in inflammatory pain. This conclusion is consistent with the prediction from a systems pharmacology model of the nerve growth factor (NGF) pathway¹⁶ utilizing PF-06273340 data. Further studies are needed to determine how these pharmacodynamic effects in a healthy volunteer study relate to the exposures needed in patients with a chronic pain condition.

The current study has demonstrated for the first time that a pan Trk molecule can reduce hyperalgesia in human subjects. The observed effect in the pre sensitised UVB assessment is consistent with the observed efficacy of anti-NGF monoclonals in chronic pain states with an inflammatory component such as osteoarthritis. We did not see a significant impact on the cold pressor or electrical stair tests where pregabalin was shown to be effective. This may indicate the pan Trk mechanism will be less effective in neuropathic pain states where pregabalin has proven efficacy; however there is uncertainty regarding the translation of studies in healthy human subjects to those with chronic pain conditions and it should be noted that the anti-NGF monoclonals previously showed efficacy in neuropathic pain,¹⁷ albeit at higher doses and exposures than in the inflammatory pain state of OA. Another uncertainty is how to interpret the effect size seen with the top dose of PF-06273340, which was similar to that observed with ibuprofen. We regard the primary role of the human evoked pain models as an early demonstration

of pharmacodynamics for novel molecules and to provide some guidance as to which pain states might be selected for future clinical studies. Optimism than pan Trk inhibitors may be more efficacious than non-steroidal anti-inflammatory drugs such as ibuprofen comes from the data with tanezumab showing superior efficacy to naproxen in OA.² Given that the methodology in this study was limited by use of single doses in a healthy subject population the translation of effect sizes to long term dosing in chronic pain states is uncertain, and will need to take account of physiological responses such as changes to nociceptors and signalling pathways brought about by chronic stimulation.¹⁸

A concern of the anti-NGF monoclonal antibodies is the increased risk of rapidly progressing osteoarthritis (RPOA), which for tanezumab monotherapy ranges from 0 events per 1,000 patient-years at 2.5 mg dose to 11 events per 1,000 patient-years at a 10 mg dose.¹⁹ Small molecule pan Trk inhibitors have the potential advantages of greater flexibility in dosing and that once dosing is stopped the drug will be rapidly eliminated compared to the much slower clearance of a humanised monoclonal antibody. Whether the risk of RPOA is reduced by small molecule pan Trk inhibitors is unknown. Given the low frequency of RPOA in subjects who received anti-NGF monoclonals an assessment of this risk must await larger clinical trials, unless a predictive nonclinical model of RPOA becomes available. We have seen no safety concerns for joint damage to date in our phase 1 programme.

In summary the current study confirms the usefulness of the human evoked pain models to profile novel pain therapeutics in early clinical development. The pan Trk inhibitor PF-06273340 demonstrated a significant effect in the UVB heat PDT assessment providing good evidence of a translation of nonclinical effects into man. This human pain model is easy to execute due to a small sample size in healthy subjects, and we believe it provides a powerful method for demonstrating an analgesic effect for novel pain medications.

REFERENCES

- Rukwied R, Mayer A, Kluschina O, Obreja O, Schley M, Schmelz M. NGF induces non-inflammatory localized and lasting mechanical and thermal hypersensitivity in human skin. *PAIN*. 2010 Mar 31;148(3):407-13.
- Ekman EF, Gimbel JS, Bello AE, Smith MD, Keller DS, Annis KM, Brown MT, West CR, Verburg KM. Efficacy and safety of intravenous tanezumab for the symptomatic treatment of osteoarthritis: 2 randomized controlled trials versus naproxen. *The Journal of rheumatology*. 2014 Nov 1;41(11):2249-59.
- Gimbel JS, Kivitz AJ, Bramson C, Nemeth MA, Keller DS, Brown MT, West CR, Verburg KM. Long-term safety and effectiveness of tanezumab as treatment for chronic low back pain. *PAIN*. 2014 Sep 30;155(9):1793-801.
- Pezet S, McMahon SB. Neurotrophins: mediators and modulators of pain. *Annu. Rev. Neurosci.* 2006 Jul 21;29:507-38.
- Nijs J, Meeus M, Versijpt J, Moens M, Bos I, Knaepen K, Meeusen R. Brain-derived neurotrophic factor as a driving force behind neuroplasticity in neuropathic and central sensitization pain: a new therapeutic target? Expert opinion on therapeutic targets. 2015 Apr 3;19(4):565-76.
- Skerratt S, Andrews MD, Bagal SK, Bilsland J, Brown D, Bungay PJ, Cole S, Gibson KR, Jones R, Morao I, Nedderman A, Omoto K, Robinson C, Ryckmans T, Skinner K, Stuppel P, Waldron G. The Discovery of a Potent, Selective and Peripherally Restricted Pan-Trk Inhibitor (PF-06273340) for the Treatment of Pain. *J Med Chem*. 2016 Nov 23;59(22):10084-10099.
- Ashraf S, Bouhana KS, Pheneger J, Andrews SW, Walsh DA. Selective inhibition of tropomyosin-receptor-kinase A (TrkA) reduces pain and joint damage in two rat models of inflammatory arthritis. *Arthritis research & therapy*. 2016 May 4;18(1):1.
- Hay JL, Okkerse P, van Amerongen G, de Kam ML, Groeneveld GJ. The use of a battery of pain models to detect analgesic properties of compounds: a two-part, four-way, randomised, placebo-controlled, crossover study. *Clinical Therapeutics*. 2015 Aug 1;37(8):e14.
- Hay JL, Okkerse P, van Amerongen G, Groeneveld GJ. Determining Pain Detection and Tolerance Thresholds Using an Integrated, Multi-Modal Pain Task Battery. *JoVE (Journal of Visualized Experiments)*. 2016 Apr 14(110):e53800.
- Kenward MG, Roger JH. The use of baseline covariates in crossover studies. *Biostatistics (Oxford, England)*. 2010 Jan;11(1):1-7.
- Southan C, Sharman JL, Benson HE, Faccenda E, Pawson AJ, Alexander SPH, et al. The IUPHAR/BPS guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. *Nucleic Acids Res* 2016; 44: D1054-68.
- Alexander SPH, Fabbro D, Kelly E, Marrión N, Peters JA, Benson HE, et al. The Concise Guide to PHARMACOLOGY 2015/16: Catalytic receptors. *Br J Pharmacol* 2015; 172: 5979-6023.
- Saadé NE, Nasr IW, Massaad CA, Safieh-Garabedian B, Jabbur SJ, Kanaan SA. Modulation of ultraviolet-induced hyperalgesia and cytokine upregulation by interleukins 10 and 13. *British journal of pharmacology*. 2000 Dec 1;131(7):1317-24.
- Weinkauff B, Rukwied R, Quiding H, Dahllund L, Johansson P, Schmelz M. Local gene expression changes after UV-irradiation of human skin. *PLoS One*. 2012 Jun 22;7(6):e39411.
- Mantyh PW, Koltzenburg M, Mendell LM, Tive L, Shelton DL. Antagonism of nerve growth factor-TrkA signaling and the relief of pain. *The Journal of the American Society of Anesthesiologists*. 2011 Jul 1;115(1):189-204.
- Ji RR, Samad TA, Jin SX, Schmolz R, Woolf CJ. p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron*. 2002 Sep 26;36(1):57-68.
- Khan N, Smith MT. Neurotrophins and neuropathic pain: role in pathobiology. *Molecules*. 2015 Jun 9;20(6):10657-88.
- Benson N, Matsuura T, Smirnov S, Demin O, Jones HM, Dua P, van der Graaf PH. Systems pharmacology of the nerve growth factor pathway: use of a systems biology model for the identification of key drug targets using sensitivity analysis and the integration of physiology and pharmacology. *Interface focus*. 2013 Apr 6;3(2):20120071.
- Bramson C, Herrmann DN, Carey W, Keller D, Brown MT, West CR, Verburg KM, Dyck PJ. Exploring the role of tanezumab as a novel treatment for the relief of neuropathic pain. *Pain Medicine*. 2015 Jun 1;16(6):1163-76.
- Turk DC1, Wilson HD, Cahana A. Treatment of chronic non-cancer pain. *Lancet*. 2011 Jun 25;377(9784):2226-35.
- Hochberg MC, Tive LA, Abramson SB, Vignon E, Verburg KM, West CR, Smith MD, Hungerford. When Is Osteonecrosis Not Osteonecrosis?: Adjudication of Reported Serious Adverse Joint Events in the Tanezumab Clinical Development Program. *Arthritis & Rheumatology*. 2016 Feb 1;68(2):382-91.

Table 1 – Summary of demographic and baseline characteristics

All subjects		
	Number of subjects	20
SEX		
	Male	20
	Female	0
AGE (YEARS)		
	< 25	11
	25–44	9
	> 45	0
	Mean (sd)	26.0 (6.9)
	Range	18–43
RACE		
	White	15
	Other	5
WEIGHT (KG)		
	Mean (sd)	77.1 (8.1)
	Range	62.7–95.4
BODY MASS INDEX (KG/M ²)		
	Mean (sd)	23.7 (2.5)
	Range	18.2–29.7
HEIGHT (CM)		
	Mean (sd)	180.4 (5.5)
	Range	169.8–190.2

sd, standard deviation

Table 2 – Summary of results for the primary analyses. PTT endpoints were analysed on the log scale, so results are presented as geometric means with %CVs for individual treatment results, and back-transformed LS mean ratios and 95% CIs for treatment comparisons.

Endpoint	Placebo (n=19; 0–4h)	Placebo (n=19; 0–6h)	Ibuprofen 600mg (n=20; 0–4h)		Pregabalin 300mg (n=19; 0–6h)		PF-06273340 50mg (n=19; 0–4h)		PF-06273340 400mg (n=19; 0–4h)	
	LSMean (90% CI)	LSMean (90% CI)	LSMean (90% CI)	Effect vs. placebo (90% CI)*	LSMean (90% CI)	Effect vs. placebo (90% CI)*	LSMean (90% CI)	Effect vs. placebo (90% CI)*	LSMean (90% CI)	Effect vs. placebo (90% CI)*
Cold	28.05	27.81	29.33	1.05	33.97	1.22	27.50	0.98	28.61	1.02
Pressor	(26.26, 29.97)	(26.00, 29.76)	(27.50, 31.28)	(0.95, 1.15)	(31.76, 36.34)	(1.11 , 1.34)	(25.74, 29.38)	(0.89, 1.08)	(26.78, 30.57)	(0.93, 1.12)
Electrical	24.36	24.60	25.00	1.03	26.92	1.09	23.66	0.97	22.98	0.94
Stair	(23.06, 25.74)	(23.21, 26.06)	(23.68, 26.38)	(0.95, 1.11)	(25.40, 28.53)	(1.01 , 1.19)	(22.39, 25.01)	(0.90, 1.05)	(21.75, 24.28)	(0.87, 1.02)
Pressure	53.36	53.35	56.77	1.06	57.06	1.07	53.63	1.01	56.08	1.05
Pain	(50.66, 56.19)	(50.59, 56.25)	(53.98, 59.69)	(0.99, 1.14)	(54.11, 60.18)	(0.99, 1.15)	(50.94, 56.47)	(0.93, 1.08)	(53.27, 59.04)	(0.98, 1.13)
Normal	46.68	46.74	46.54	-0.13	47.09	0.35	46.87	0.19	46.83	0.16
Heat	(46.36, 46.99)	(46.42, 47.06)	(46.24, 46.85)	(-0.57, 0.31)	(46.76, 47.41)	(-0.11, 0.81)	(46.55, 47.18)	(-0.26, 0.64)	(46.52, 47.15)	(-0.29, 0.60)
UVB	40.71	40.72	42.10	1.39	41.18	0.47	40.98	0.27	41.84	1.13
Heat	(40.37, 41.05)	(40.37, 41.06)	(41.77, 42.43)	(0.91 , 1.87)	(40.84, 41.53)	(-0.02, 0.95)	(40.63, 41.33)	(-0.22, 0.76)	(41.49, 42.18)	(0.64 , 1.61)

ci, confidence interval; cv, coefficient of variation; gm, geometric mean; lsmeans, least squares mean; pdt, pain detection threshold; prt, pain tolerance threshold; sd, standard deviation; uvb, ultraviolet B Bold text indicates that the effect over placebo met the predefined decision criterion

Table 3 – Descriptive summary of plasma PF-06273340 pharmacokinetic parameter values.

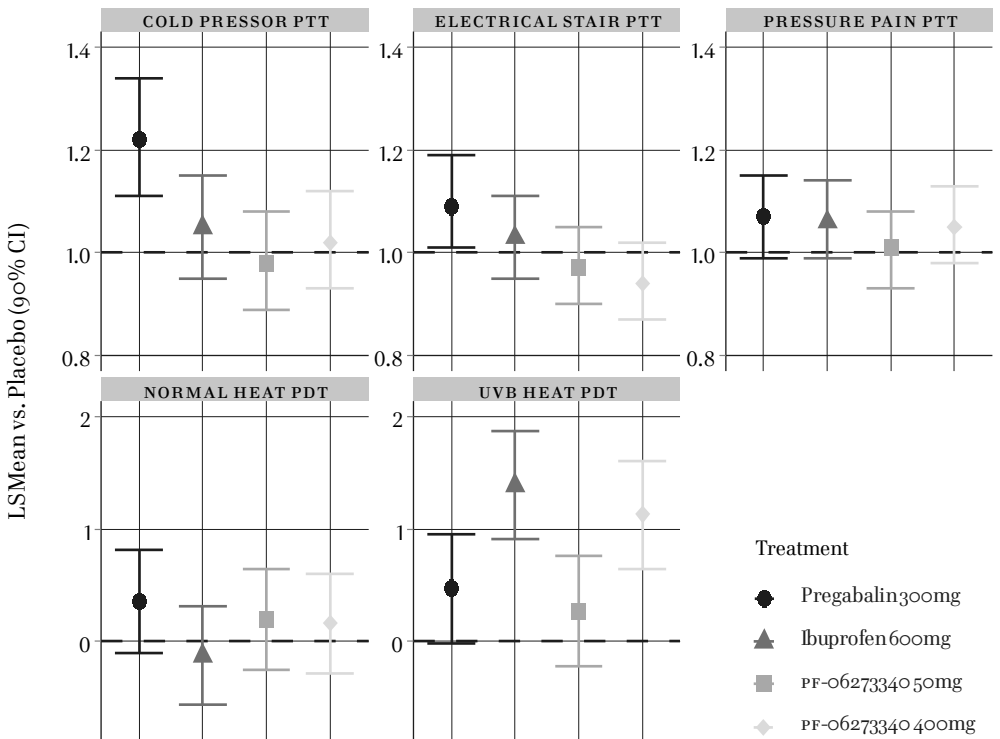
Parameter summary statisticsa by PF-06273340 treatment		
Parameter (units)	400 mg	50 mg
N	19	19
AUClast (ng•hr/mL)	3630 (43)	483.5 (42)
cmax (ng/mL)	1396 (56)	150.4 (57)
tmax (hr)	1.00 (0.500–2.00)	1.08 (1.00–3.00)

AUClast, area under the plasma concentration–time curve from time 0 to the time of last quantifiable concentration; cmax, maximum observed plasma concentration; %cv, percentage coefficient of variation; tmax, time to reach cmax
a Geometric mean (geometric %cv) for all except: median (range) for tmax.

Figure 1 – Disposition of subjects.

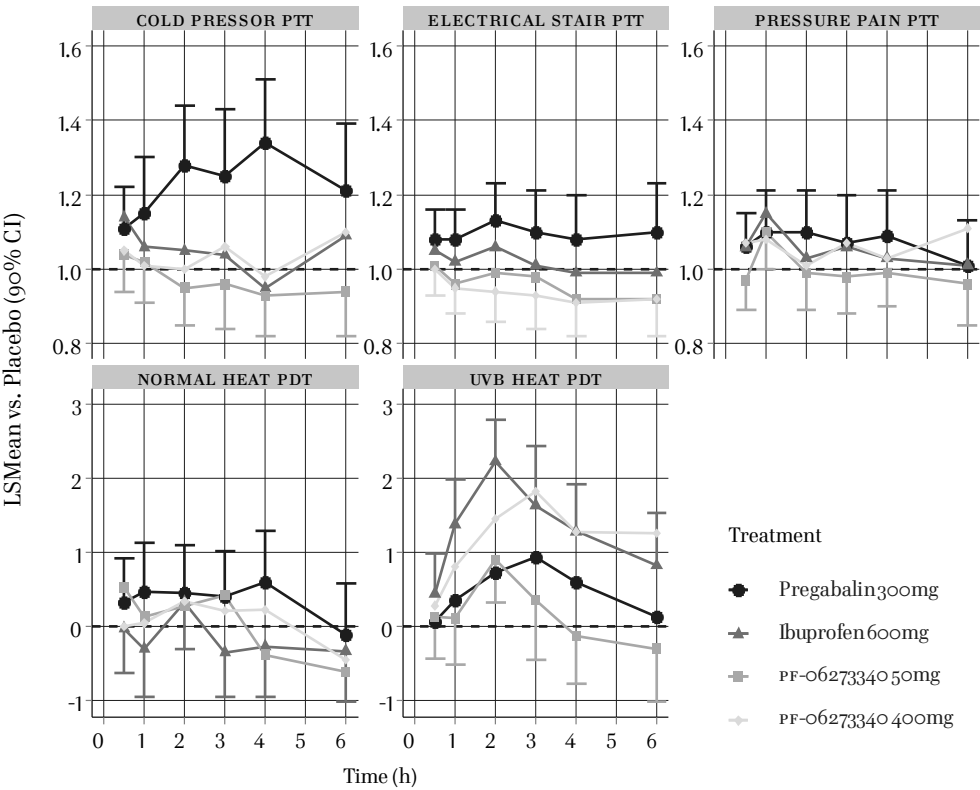
	Randomized (n = 20)						
Allocation	Allocation to treatment sequence (n = 20)						
	Received allocated intervention:						
	Treatment	Period					Total
		1	2	3	4	5	
	Placebo	n = 4	n = 4	n = 4	n = 3	n = 4	n = 19
	Pregabalin 300 mg	n = 4	n = 4	n = 4	n = 4	n = 3	n = 19
	Ibuprofen 600 mg	n = 4	n = 4	n = 4	n = 4	n = 4	n = 20
Follow up	PF-06273340 50 mg	n = 4	n = 4	n = 4	n = 4	n = 3	n = 19
	PF-06273340 400 mg	n = 4	n = 3	n = 4	n = 4	n = 4	n = 19
	Completed the study (n = 18)						
	Discontinued:						
Analysis	Analysed (n = 18)						
	• Excluded from primary analysis (n=0)						

Figure 2 – Primary analysis results. The comparisons of PF-06273340 vs. placebo, and ibuprofen vs. placebo were made with LS means averaged over 4 hours. The comparison of pregabalin vs. placebo was made with LS means averaged over 6 hours. The purple horizontal dashed line represents no effect over placebo. PTT endpoints are presented on the fold-change to placebo scale, whereas PDT endpoints are presented on the absolute difference to placebo scale.



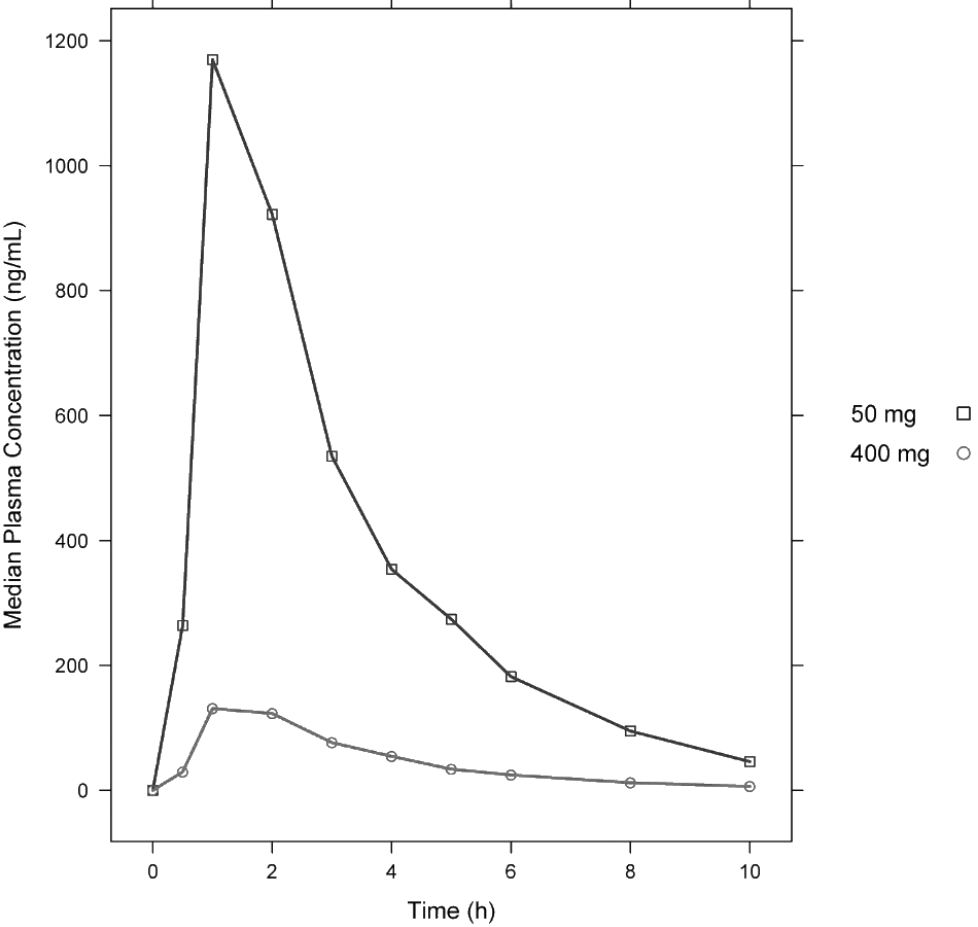
CI, confidence interval; LS means, least squares mean; PDT, pain detection threshold; PTT, pain tolerance threshold; UVB, ultraviolet B

Figure 3 – Time course of treatment effects across the 5 Primary Endpoints. The dotted horizontal line represents no effect over placebo. PTT endpoints are presented on the fold-change to placebo scale, whereas PDT endpoints are presented on the absolute difference to placebo scale.



CI = confidence interval; LS means = Least Square mean; PDT = pain detection threshold; PTT = pain tolerance threshold.

Figure 4 – Median Plasma PF-06273340 Concentration-Time Profiles.



SUPPLEMENTARY INFORMATION

UVB irradiation was applied at the screening visit in ascending doses (corresponding to different irradiation times) to 6 different 1×1 cm areas of skin on the back to determine the individual UVB dose that produced the first clearly discernible erythema. The 3-fold individual minimal erythema dose (MED) of UVB was applied 24 hours prior to dosing to the subject's back to produce local cutaneous inflammation, thereby inducing a homogeneous area of skin erythema and hyperalgesia. The area of skin irradiated was 3×3 cm. Subsequently, a 3×3 cm thermode was used to measure pain detection thresholds (initially 34°C , ramp 0.5°C/s , average of 3 stimuli) on the normal skin contralateral to the site of UVB irradiation and on the UVB irradiated skin (Cutoff 50°C).

For electrical pain electrodes were placed on skin, 10 cm distal from the patella overlying the tibia. Electrical resistance between electrodes was to be less than 2 k Ω . For the single stimulus current intensity increased from 0 mA in steps of 0.5 mA·s⁻¹ to a maximum of 50 mA.

For the mechanical pressure pain test, an 11 cm wide tourniquet cuff was placed over the gastrocnemius muscle with a constant pressure rate increase of 0.5 kPa/s. The pneumatic pressure was increased until the subject indicated maximum pain tolerance using the eVAS slider, or a maximum pressure of 100 kPa was achieved.

For the cold pressor test, subjects placed their non-dominant hand into a water bath at $35 \pm 0.5^{\circ}\text{C}$ for 2 minutes. At 1 minute 45 seconds a blood pressure cuff on the upper arm was inflated to 20 mmHg below resting diastolic pressure. At 2 minutes the subject then moved that hand from the warm water bath, directly into a similar sized water bath at $1.0 \pm 0.5^{\circ}\text{C}$. The subjects were instructed to indicate when pain detection threshold was reached (first change in sensation from cold non-painful to painful) as well as the pain intensity, by moving the eVAS slider. When pain tolerance or a time limit (120 s) was reached, subjects were instructed to remove their hand from the water, at which point the blood pressure cuff deflated.

In the single ascending dose study (B5261001) doses up to 450 mg PF-06273340 as single doses were well tolerated. Although dose was not escalated beyond 450 mg due to 2 events of symptomatic orthostatic hypotension. There were no deaths, serious adverse events (SAEs), severe AEs, dose reductions or discontinuations due to AEs at this dose level. There were no clinically significant changes in laboratory signs, ECGs or physical examination observed at doses up to 450

mg single dose. In a subsequent bioavailability study B5261003, up to 12 subjects received different formulations of 400 mg PF-06273340 on 5 occasions. Dosing was well tolerated with no SAEs, severe AEs, significant abnormalities in laboratory parameters, pulse rate or ECG. All AEs were mild apart from one moderate AE of upper respiratory tract infection. No subject experienced symptomatic orthostatic hypotension. Although several subjects experienced asymptomatic episodes. In multiple dose study B5261002 in healthy young and elderly subjects received doses of 100 mg or 200 mg 3 times a day (TID). The most common AEs were myalgia and orthostatic hypotension. Four subjects discontinued due to AEs: 1 subject on Day 3 due to moderate hypertension, 1 subject withdrew on Day 5 due to moderate orthostatic hypotension, and 2 were withdrawn by the sponsor on Days 10 and 13 due to elevations in ALT. Monitoring continued and ALT returned to normal range by Day 22 in both subjects. There were no notable changes in bilirubin (total, direct or indirect) over this time period, and were no Hy's Law cases. A dose of 100 mg TID was considered maximum tolerated dose and was well tolerated with no changes in Liver Function Tests (LFTs) above ULN. The incidence of symptomatic and asymptomatic orthostatic hypotension was similar to that seen with placebo.