Randomized Trial

Randomized Clinical Trial Evaluating Transdermal Ibuprofen for Moderate to Severe Knee Osteoarthritis

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Background: Osteoarthritis is a common condition, typically treated with orally administered analgesics and non-steroidal anti-inflammatory drugs (NSAIDs). Chronic administration of NSAIDs, serotonin-norepinephrine reuptake inhibitors (SNRIs, i.e., duloxetine), and opioid medications (i.e., tramadole) is regularly associated with multiple, serious side effects, in part due to the route of administration. Transdermal delivery of NSAIDs, such as ibuprofen, represents a potentially alternative treatment for this inflammatory pain condition with a better therapeutic profile.

Objective: Investigate the safety and efficacy of a novel transdermal ibuprofen formulation (VALE®-ibuprofen) containing 10% ibuprofen, compared to a placebo in a randomized, double-blinded clinical trial, for clinical improvement in patients with moderate to severe painful osteoarthritis of the knee.

Study Design: A randomized, placebo-controlled, double blind, multi-center Phase 2 clinical trial.

Setting: An academic medical center, and private rheumatology and interventional pain management practices in Massachusetts and in Switzerland.

Methods: The Phase 2 clinical study included patients with primary osteoarthritis in a single knee joint with a progression level of moderate to severe based in part on a grade II or III designation according to the Kellgren and Lawrence classification system. Patients received the corresponding, randomly assigned study formulation (VALE-ibuprofen or placebo) for application to the target knee at a dose of 2.0 grams of drug product (200 mg ibuprofen) twice daily for 14 days. The evaluation of the efficacy of the treatments utilized the widely accepted methods of the Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index and the Visual Analog Scale (VAS) scores for the patients.

Results: The results indicate that the transdermal VALE-ibuprofen formulation was very well tolerated from a safety perspective during the 2-week trial and also produced significant, positive clinical improvements superior to the placebo in all clinical endpoints tested. In particular, the $WOMAC_{Total}$ and $WOMAC_{Physical}$ Functioning, for the VALE-ibuprofen, were superior compared to the placebo ($P = 0.0283$ and $P = 0.0201$, respectively). Other clinical endpoints including the $WOMAC_{Pain}$, $WOMAC_{Stiffness}$, and $VAS_{Resting}$ scores were superior to those obtained from the placebo group, trending towards statistical significance compared to placebo ($P = 0.0811$, 0.1103, and 0.0785, respectively). Based on the Patient and Physician Global Impression of Change survey, patient satisfaction slightly improved across both groups; however, no statistical significance was detectable as compared to the baseline.

Limitations: The sample size of 64 subjects in the final data analysis and the lack of including an orally administered drug group are limitations of this study.

Conclusions: The use of transdermal VALE-ibuprofen has beneficial clinical effects on the pain levels experienced in some patients with moderate to severe osteoarthritis of the knee as measured by the WOMAC Osteoarthritis Indices for stiffness, pain, physical function, and total. Visual Analog Scales (VAS) tests, VAS_{Motion} and $VAS_{Weight\text{-}bearing}$, again while appeared superior to placebo, were not statistically different from placebo.

Clinical Trial Registration: NCT01496326

Key words: Ibuprofen, transdermal treatment, VALE-ibuprofen, knee osteoarthritis, Visual Analog Scale, WOMAC score

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Osteoarthritis (OA) is a common disease in subjects aged 50 years and older. Approximately 33% of those aged 63 to 93 (27% of age ≤ 70 and 44% of those ≥ 80) have radiologic evidence of knee OA and suffer from knee pain, half of whom have severe difficulty with physical function or pain (1-3). While OA has been considered a complex arthropathy in which the cartilage destruction and bone damage are the hallmarks of the disease, inflammation is a significant factor associated with the progression of cartilage loss. Symptoms of the disease including joint pain, swelling, and stiffness are indicators of synovitis (4,5). Analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), duloxetine, tramadole, and diclofenac gel are the most commonly prescribed medications for knee pain (6-9).

Oral NSAIDs are associated with safety risks including gastrointestinal side effects (10-14), renal insufficiency (15,16), hepatic toxicity, exacerbation of asthma, sodium retention, raised blood pressure, and resistance to anti-hypertensive drugs, as well as increased risk of thrombotic cardiovascular events (17-19) for non-aspirin agents and increased risk of intracerebral hemorrhage and other bleeding with aspirin. To avoid the above listed side effects of treatment with NSAIDs, mainly gastrointestinal side effects, agents have been developed that selectively inhibit type 2 cyclooxygenase (COX-2) rather than type 1 cyclooxygenase (COX-1). However, in some examples, due to severe cardiovascular side effects, several novel COX-2 inhibitors have been withdrawn from the market (14,18).

Transdermal NSAIDs directly delivered to the site of the OA is an attractive alternative to oral NSAIDs with the potential advantage of improving the safety profile as well as improving the efficacy, in particular on the potential of a daily dose basis of the active pharmacological ingredient (20-26). The pain-relieving efficacy of topical formulations of ibuprofen has been compared to oral ibuprofen (23,27-31). These results documented that topically applied ibuprofen can be safe and effective in the treatment of knee OA. The risks of gastrointestinal side effects for high-dose (1125 mg per day) topically applied ibuprofen were comparable with either low-dose (≤ 1200 mg per day) ibuprofen or with COX-2 inhibitors (10,21,28,32-34).

Methods

Study Design

This randomized, double-blind, placebo-controlled, multi-center trial was designed based on Consolidated Standards of Reporting Trials (CONSORT) (35). The study was carried out in accordance with Good Clinical Practice as issued by the following guidelines: (i) ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996 (CPMP/ICH/135/95); (ii) Declaration of Helsinki, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects, Helsinki 1964, amendments Tokyo 1975, Venice 1983, Hong Kong 1989, South Africa 1996, and Edinburgh 2000); (iii) Swiss Federal Law on Medicinal Products and Medical Devices and related ordinances, dated December 15, 2000 (Bundesgesetz über Arzneimittel und Medizinprodukte, incl. Ausführungsbestimmungen); (iv) Ordinance on Clinical Trials of Therapeutic Products, dated October 17, 2001 (Verordnung über klinische Versuche mit Heilmitteln [Vklin]). The study was approved by the Institutional Review Board, was registered with the U.S. Clinical Trial Registry (NCT01496326), and was conducted at a major academic medical center as well as in rheumatology and interventional pain management practices in Switzerland. Participants were recruited from established patients and new patients responding to advertisements and presenting to the clinical centers.

This trial was conducted with a patient population with primary OA in a single knee joint with a severity level scaled as grade II or III based on the Kellgren and Lawrence classification system. Enrolled subjects were randomly allocated to be treated twice a day with topical ibuprofen or its placebo for 14 days. After the initial clinic screening established that the patient met the inclusion and exclusion criteria, the patients were randomized for entry into the clinical trial to receive either the ibuprofen or the placebo.

Study Population

A total of 75 patients with radiologically confirmed and symptomatically active grade II or III moderate to severe knee OA who satisfied the clinical protocol inclusion and exclusion criteria were randomized and entered the clinical trial (36-38). The inclusion criteria included males or females > 40 years old, a Visual Analog Scale (resting) score of ≥ 40mm on a 100 mm scale, an osteoarthritis radiologic score of grade II or III based on the Kellgren and Lawrence classification system, capable and willing to execute an informed consent document to enter and comply with the protocol (Table 1). Patients were excluded from participating in the trial according to the clinical protocol for reasons includ-

Table 1. *Study population demographics and baseline characteristics.*

Inclusion Criteria	Exclusion Criteria
Male or female patients aged at least 40 years.	Concomitant presence of another type of continuous pain that is more severe in intensity in comparison with the osteoarthritis target joint pain (e.g. low back pain, fibromyalgia, ankylosing spondylitis, etc.).
Generally good health as confirmed by medical and previous medication history, and baseline physical examination.	Osteoarthritis causing significant pain in any joint other than the identified knee, i.e., pain in hip, back, or contralateral knee (≥ 20 mm pain) as confirmed by a separate VAS at visit 1 for any other painful joint concerned.
Body mass index between 20.0 and 32.0 kg/m^2.	Concomitant therapies interfering with the study objectives, including: arthrocentesis or arthroscopic techniques within 3 months prior to the study; administration in the target joint of intra- or periarticular corticosteroid injections within 6 weeks of study entry or hyaluronan injections within 6 months of study entry; treatment with a strong opioid in the 4 weeks preceding study entry; subjects taking NSAIDs, COX-2 selective inhibitors or steroidal drugs for less than 4 weeks before study entry; subjects taking NSAIDs, COX-2 selective inhibitors or steroidal drugs for more than 4 weeks may continue these medications during the study; however, at a stable and constant dosage for at least 2 weeks before study entry and throughout the study.
Postmenopausal conditions for female subjects for at least 2 years, Patients with primary osteoarthritis in a single knee joint, grade II or III (Kellgren and Lawrence classification).	Major surgery in the 3 months preceding the study.
Radiographic evidence consistent with osteoarthritis carried out within 6 months before screening.	Female patients who are pregnant or breast-feeding.
The pain suffered by the patient is currently not adequately controlled with a simple analgesic or an NSAID or would necessitate treatment, but is not yet treated. This will be defined as a pain control assessment of "poor" or "very poor" and a mean pain at rest score ≥ 40 (on a VAS of 0–100 mm) at Visit 1.	Known presence of any of the following: Clinically significant abnormality in clinical laboratory tests at Visit 1 as determined by the Investigator or designee. In case a laboratory value exceeds the 1.5 x upper limit of normal (ULN). Any disease or condition that compromises the function of those body systems that could result in altered absorption, excess accumulation, or impaired metabolism or excretion of the test medications. A life-threatening disease that would preclude completion of study or interfere with protocol compliance; Significant psychiatric disorder (including major depression) or subjects receiving anti-psychotic medication.
	History of serious adverse reactions or hypersensitivity to any drug requiring therapy, including intrinsic asthma.
	Presence or history of allergies requiring acute or chronic treatment (except e.g. seasonal allergic rhinitis).
	Previous discontinuation of a topical ibuprofen treatment due to adverse events. History of alcohol or drug abuse in the last 5 years, or documented or suspected history of an addictive personality.
	Subjects who have received an investigational drug or have used an investigational medical device in the 30 days preceding study entry.

ing experiencing non-target knee pain associated with non-arthritic pain and/or other sites of OA pain above a designated level (i.e., $VAS_{Resting}$ > 20mm/100mm), administering certain concomitant medications (i.e., steroids, narcotics, pain-relievers, NSAIDS, sedatives, or muscle relaxants and other listed medications) within a period of time before entering the trial, experienced major surgery within 3 months preceding the trial, pregnant or breast feeding, presence of an active infection or psychiatric disorder (Table 1).

Subsequently, of the 75 patients entering the trial (Intent-to-Treat group), 71 patients completed the trial. Prior to un-blinding the database, a post-trial assessment of the patient profiles and the patient's compliance with the protocol was conducted (Fig. 1). It was determined that 7 additional patients had not sufficiently complied with the protocol requirements or had not initially met the inclusion and exclusion criteria and they were subsequently not included in the final analysis (final analysis group, Per-Protocol group, Efficacy population, n = 64). The CONSORT flow diagram of the trial and patient population is shown in Fig. 1. Of the 7 patients who completed the trial and were not included in the trial analysis, 3 VALE®-ibuprofen

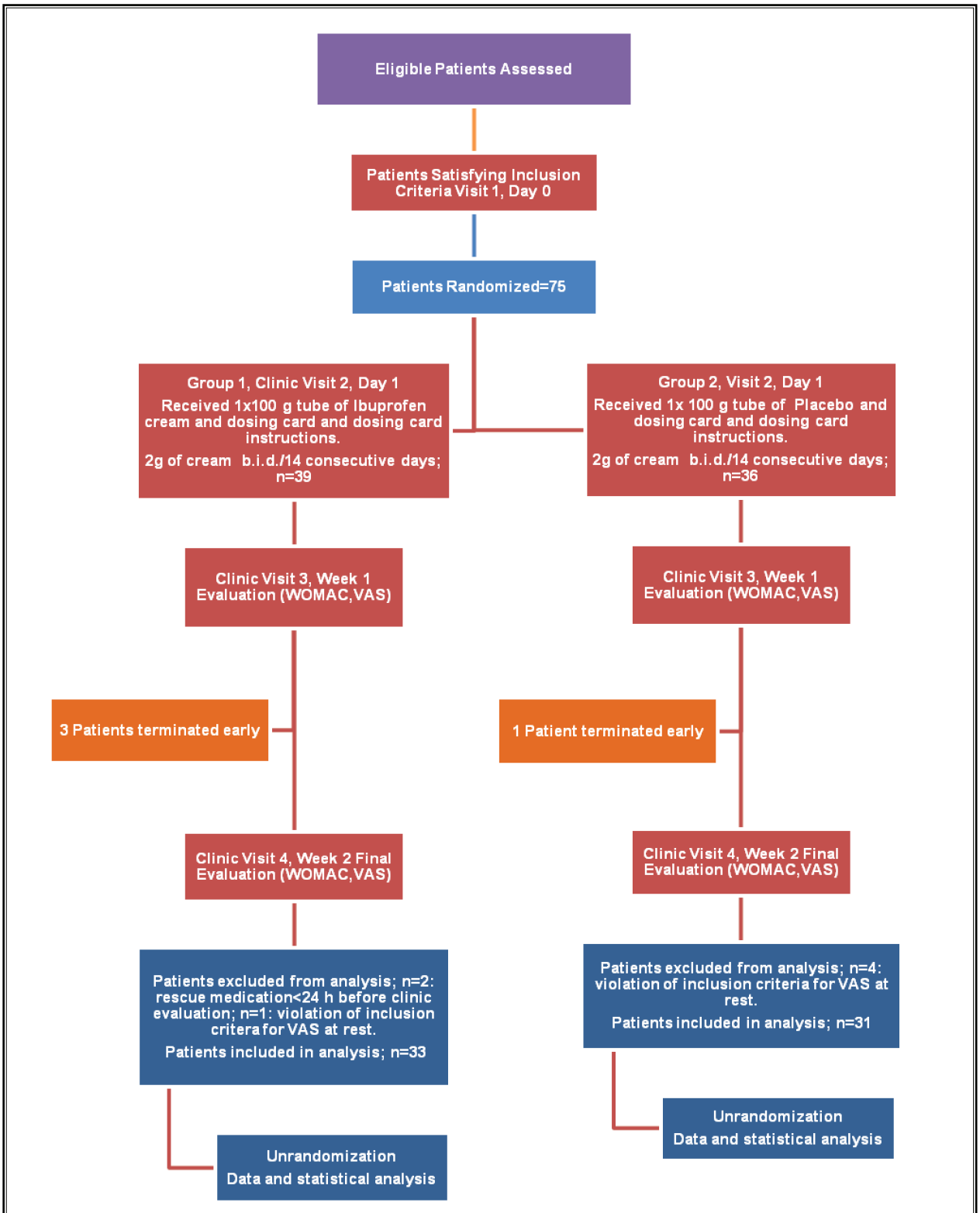


Fig. 1. *Schematic presentation of study and patient population flow.*

and 4 placebo patients were not included for violating the protocol by taking rescue medication within the 24 hour period before the clinic visit, or for taking incorrect dose of the medication, or taking steroid medications during the studies (Fig. 1). The 2 treatment groups were of similar age, gender composition, medication history, baseline physical exam and vital signs, and baseline severity of pain. There were no significant differences in body mass index, duration of pain, or chronic pain grade between the 2 groups (Table 2).

Treatment Intervention

The study drug ibuprofen was formulated in a cream containing 10% (w/w) ibuprofen. This novel drug delivery formulation, termed vasoactive and lipid-encapsulated (VALE) is capable of effectively delivering small molecules deep in the skin and the tissues underneath. The ibuprofen and placebo drug products were manufactured under GMP conditions by Contract Pharmaceuticals Limited-Niagara, Buffalo, New York, USA.

The clinical research organization Pharma Focus, Ltd. (PFC) (currently Clinipace), Volketswil, Switzerland, conducted and monitored the Phase 2 clinical trial. The study medication for each qualified patient was selected as either VALE-ibuprofen or placebo in a randomized process; packed and labeled for each patient by the responsible person at PFC. Investigators were randomly provided with blinded samples of either ibuprofen or placebo in 100 gram tubes to distribute the drug product for the conduct of the clinical trial.

Investigational Plan and Investigated Parameters

Patients who satisfied the clinical protocol inclusion and exclusion criteria to participate in the trial were provided with the necessary informed consent, and entered into the randomization process (Clinic Visit 1). During each subsequent clinic visit: Clinic Visit 2: trial initiation (drug product was distributed), Clinic Visit 3: intermediate assessment (Week 1), and Clinic Visit 4: trial termination (Week 2), patients completed OA and pain assessment tests including Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Indices for stiffness, pain, physical function, and total. In addition, Visual Analog Scales (VAS) tests indicating the amount of pain at rest, in motion (walking 15 meters), and with weight-bearing experienced that day and a Physician and Subject Global Impression of Change scales (7-point scale) were completed.

Patient Randomization

This study was performed under double blind conditions according to blinding and bias-reducing procedures as follows. Each subject was assigned to a 4-digit subject number in sequence of the study entry per clinic. The logic of number assignments was as fol-

Table 2. *Descriptive statistics of patient's clinical parameters by treatment group and clinic visit.*

	Ibuprofen Group	Placebo Group	*P*-value
Gender			
Male	15	12	NA
Female	24	24	NA
Age (years)*	60.8 ± 11.6	61.8 ± 11.0	0.708 (NS)
Medical and medication history**	Recorded	Recorded	NA
Vital signs : Blood pressure (mmHg Syst/diast/pulse)			
Visit 1	137/82/73 (± 14/10/8)	135/80/70 (± 12/8/10)	0.545 – 0.097*** (NS)
Visit 4	136/80/72 (± 14/12/10)	134/80/70 (± 12/9/10)	0.831 – 0.218*** (NS)
Body mass index	28.5 ± 2.9	27.4 ± 3.2	NS
Postmenopausal conditions	NA or Negative	NA or Negative	NA
Osteoarthritis Grade II and III****	Yes	Yes	NA

*Average age of study population: 61.3 years
**Medical history was recorded for all patients in the study and assessment was done according to the Inclusion/Exclusion criteria.
***The lower and upper limits of the P interval are provided. Significance is calculated by Student's t test (two tailed) and corresponding parameters between Visit 1 and Visit 4 are compared.
****Assessment is based on radiological observation.

lows: (i) first and second digit: number of the study clinic (i.e., clinic 01, clinic 02), (ii) third and fourth digit: patient's number in that clinic (i.e., 01, 02), (iii) only subjects receiving an administration of study drug received a randomization number according to a randomization list, and (iv) subjects were randomized to be treated either with VALE-ibuprofen cream or placebo. A randomization list (in blocks of 4 subjects, 2 actives and 2 placebos) were produced by the Biometric Department of PFC and a randomization number was assigned to each patient receiving study drug. A certain number of blocks were assigned to each center. Once a patient of a block was treated, the block could not be reassigned. In the event of discrepancies in the recruitment rate of the respective centers, full blocks of 4 randomization numbers and its corresponding medications might have been reassigned to a different center.

The investigators were provided with code breaker documentation for their respective patients, containing the identity of the treatment, to be opened only in case of a medical emergency. A copy of the sealed code envelopes was filed at PFC and another in the clinic of the investigators, in a secure, locked place. The integrity of these the code breaker documents was checked by the clinic monitor at the end of the study. For the overall study, the code was broken only after the study had been completed, all queries resolved, and the data base had been locked.

During Clinic Visit 2, patients received the corresponding, randomly assigned drug product, sufficient for the completion of the 14-day trial, as a single 100 gram tube of either 10% VALE-ibuprofen cream or the placebo. The dose of the cream was determined using the "ribbon" method measuring a 2-gram dose of the drug product expressed from the tube onto a calibrated line on a dosing card. The drug product was administered twice daily (b.i.d.) for 14 days.

Rescue medication: If the patient required, supplementary analgesic medication was provided (paracetamol, 500 mg tablets), to be taken during the study on an as needed basis; however, the total daily dose of paracetamol was not to exceed 2 g (4 x 500 mg tablets) for 3 consecutive days. Subject use of paracetamol was recorded in the patient diary on a daily basis. Patients were required to refrain from taking paracetamol within 24 hours of clinic visits for efficacy evaluations.

Outcome Measures

During Clinic Visits 2, 3, and 4 (Weeks 0, 1, and 2) subjects were evaluated for the efficacy of the treatment and an assessment was completed on the safety and tolerance of the patient to the treatments. The evaluation required the patient to complete WOMAC osteoarthritis pain index tests for stiffness, physical function, pain, and total (31) using a validated German translation (39). In addition, the patients completed VAS tests indicating the amount of pain experienced at rest, in motion, and with weight bearing that day. VAS is a measurement instrument that uses a linear scale to quantify the amount of pain a patient feels ranging across a spectrum of pain levels ranging from no pain (0) to extreme pain (10). Typically, the VAS is a horizontal line, 100 mm in length, with an indication of no pain on the left side of the line and extreme pain on the right side of the line and the patient marks on the line the point that they feel represents their level of pain and discomfort under that particular circumstance. Also, the patients and the physicians were asked to complete Physician and Subject Global Impression of Change scales evaluation. As a safety follow-up (Visit 5, 7 days after the last application of the study medication), the patient was contacted by phone and interviewed for a documentation of their well-being.

For local skin tolerability to the drug products, the investigator graded the status of the skin of the target knee by visual inspection using a clinical grading scale at all study visits.

Statistical Analysis

Descriptive statistics were calculated for all background variables that include median, arithmetic mean, standard deviation, 95% confidence range, maximum and minimum values (36-38).

The improvement in the VAS scores for pain: at rest, weight-bearing, and while walking 15 meters were measured by a VAS of 100 mm and the differences were calculated by subtracting the baseline (Clinic Visit 2) from the Clinic Visit 3 (Week 1) or from Clinic Visit 4 (Week 2) values. The results were compared between groups by ANOVA with generation of 95% confidence intervals for the difference VALE-ibuprofen - placebo.

For the WOMAC tests, separate results were determined for pain, stiffness, physical functioning, and total scores. Significance of differences between baseline (Clinic Visit 2) and Clinic Visits 3 and 4 were assessed using ANOVA with the generation of 95% confidence for each test score.

Global Impression of Change scores provided by investigators and by patients were tabulated according

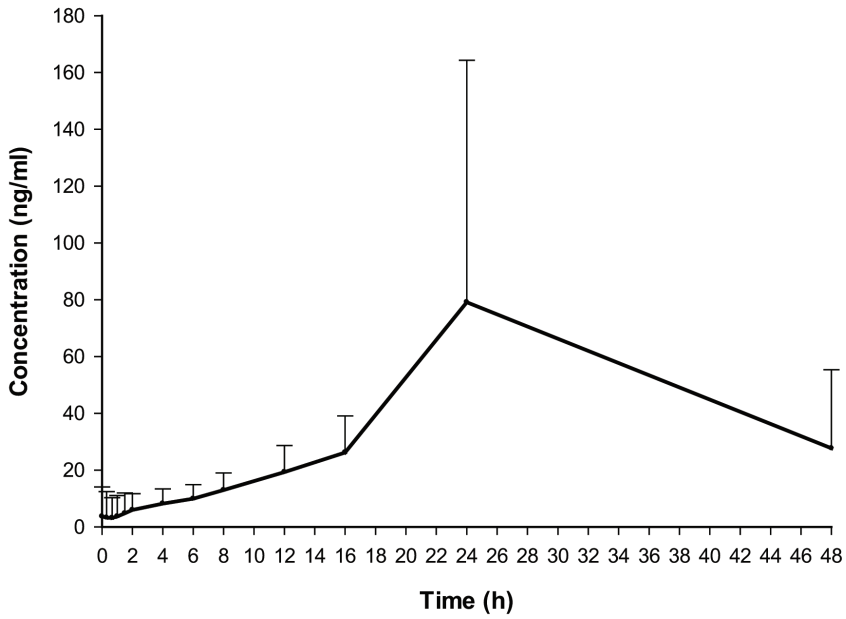


Fig. 2. *Plasma concentration of ibuprofen in human subjects after a single-dose administration of VALE-ibuprofen.*

Depicted is the pharmacokinetic profile of the arithmetic mean ± SD of the ibuprofen plasma concentration vs. time profile following a single topical application of the study formulation of VALE-ibuprofen (200 mg ibuprofen).

to the clinic visit and the treatment group and compared using Mann-Whitney's U test. All analyses were performed in SAS (40).

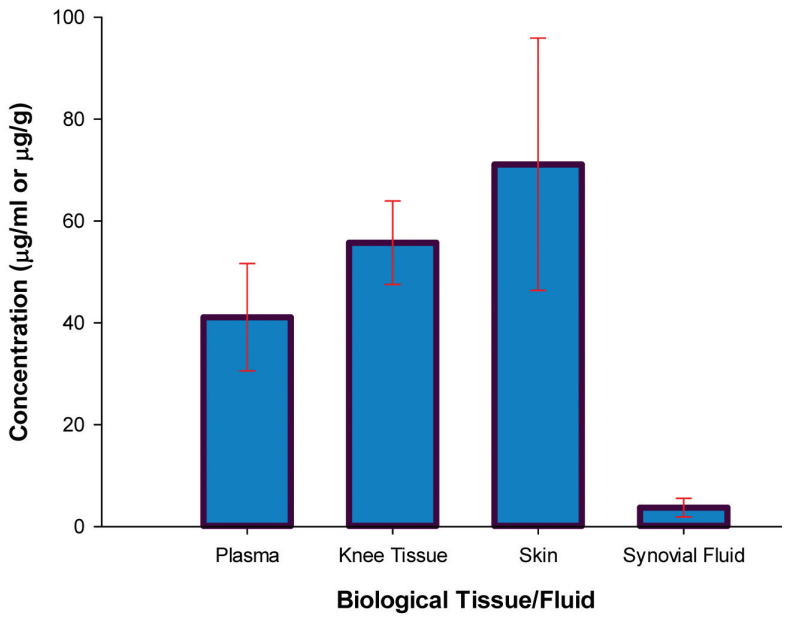
Results

Before performing the Phase 2 clinical trial, a Phase 1 safety and pharmacokinetic trial was conducted on 12 healthy volunteers at the clinic of Pharma Contract, Ltd., Allschwil (Basel), Switzerland (currently Covance Ltd.). The subjects were administered a single, 2-gram topical dose of VALE-ibuprofen (200 mg ibuprofen) or a placebo. Blood samples were analyzed for the presence of ibuprofen (41) in each of the study subjects over a 72 hour period as well as for standard blood chemistries in the evaluation of safety profiles. The study indicated no clinically significant deviations of any laboratory parameters, and there were no significant drug-related adverse events. The pharmacokinetic results indicated that there was a peak of ibuprofen present in the plasma at 24 hours following the single 200 mg application of ibuprofen; however, the amount was very low, with an average Cmax of approximately 80 ng/ml of plasma (Fig. 2), suggesting that the bulk of the ibuprofen was in the localized tissues in and around the application site.

As a secondary, related experiment to the human pharmacokinetic analysis, since there was a small amount of drug present in the plasma, we addressed the question of tissue distribution of the ibuprofen in an animal model experiment with the same formulation (VALE-ibuprofen). In this experiment, guinea pigs were dosed on their knees, b.i.d. with 50 mg doses of the VALE-ibuprofen for 4 days. Plasma was prepared and tissues were excised, homogenized, extracted, and analyzed for ibuprofen content (41). The results, shown in Fig. 3, indicated that there was a significant amount of ibuprofen present in the tissues of the knee joint as well as the muscle tissue immediately surrounding the knee, also in the plasma and the synovial fluid.

Phase 2 Clinical Trial: Clinical Endpoint Results

The evaluation of the levels of pain and discomfort experienced by the patients before and after one or 2 weeks of treatment with the transdermal VALE-ibuprofen or placebo were determined based on the results of the series of WOMAC and VAS tests. The analysis of the WOMACTotal score comparing the VALE-ibuprofen to the placebo from the baseline Clinic Visit 2 to the score obtained after 2 weeks of b.i.d. treatments on



VALE-ibuprofen (50 mg ibuprofen per dose) was topically applied b.i.d. for 4 days onto the knees of healthy guinea pigs. On Day 5, Guinea pigs were euthanized, samples of blood, skin corresponding to the application site, synovial fluid, tendon, cartilage, and muscles surrounding the joint were removed and tissues were homogenized, extracted, and analyzed for the presence and quantitative distribution of ibuprofen.

Fig. 3. *Concentration of ibuprofen in biological tissue/fluid after topical application to guinea pigs.*

Clinic Visit 4 indicates a clinically positive effect, and additionally the effect is statistically significant (P = 0.0283) compared to the placebo (Table 2 and Fig. 4A). The improvement in this pain parameter was 116.6% greater for the VALE-ibuprofen set than the placebo set. The $WOMAC_{Physical\ function}$ evaluation demonstrated that the difference from baseline Clinic Visit 2 and Clinic Visit 4 resulted in an improvement of 147.8% for the VALE-ibuprofen over the placebo with a statistical significance by ANOVA of a P = 0.0201 (Table 2, Fig. 4B).

The $WOMAC_{Stiffness}$ indicated that the VALE-ibuprofen clinical group had a 122.6% improvement in the score over placebo (Table 2, Fig. 5A), with a trend towards statistically significant value of P = 0.1103. Comparing the same test results for $WOMAC_{Stiffness}$ between Week 1 and Week 2, the VALE-ibuprofen group continued to improve (22%) from Week 1, while in contrast, the placebo group regressed (Table 2, Fig. 5B); resulting in a robust improvement comparing the 2 clinical groups (P = 0.0201). The evaluation of $WOMAC_{Pain}$ scores between Clinic Visit 2 and Visit 4 showed a 70.1% improvement over the placebo effect, with a trend towards statistically significant value of P = 0.0811 (Table 2).

The Patient and Physician Global Impression of Change survey and patient satisfaction slightly improved across both groups; however, no statistical significance was detected as compared to the baseline (Table 3).

The evaluation of the $VAS_{Resting}$ clinical parameter between Clinic Visit 2, 3, and 4 comparing the VALE-ibuprofen clinical set and the placebo group is summarized in Table 2 and also in Figs. 6A and 6B. The VALE-ibuprofen group improved 60.6% compared to the placebo in the $VAS_{Resting}$ assessment between baseline Clinic Visit 2 and Clinic Visit 4 (Week 2) resulting in trending towards statistical significance in improvement (ANOVA analysis P = 0.0785). The $VAS_{Resting}$ pain scale assessment between Clinic Visit 3 and Clinic Visit 4 also indicated a substantial improvement compared to the placebo group (P = 0.0482).

The evaluation of the other VAS tests comparing the VALE-Ibuprofen clinical set and the placebo for VAS_{Motion} or $VAS_{Weight\text{-}bearing}$ pain scores also indicated that the VALE-ibuprofen produced superior results of improvement over the placebo; however, these tests were not statistically significant between the VALE-ibuprofen and placebo groups (Table 2).

Adverse events were noticed for one patient in the VALE-ibuprofen group and in the placebo group, with each patient reporting a mild rash.

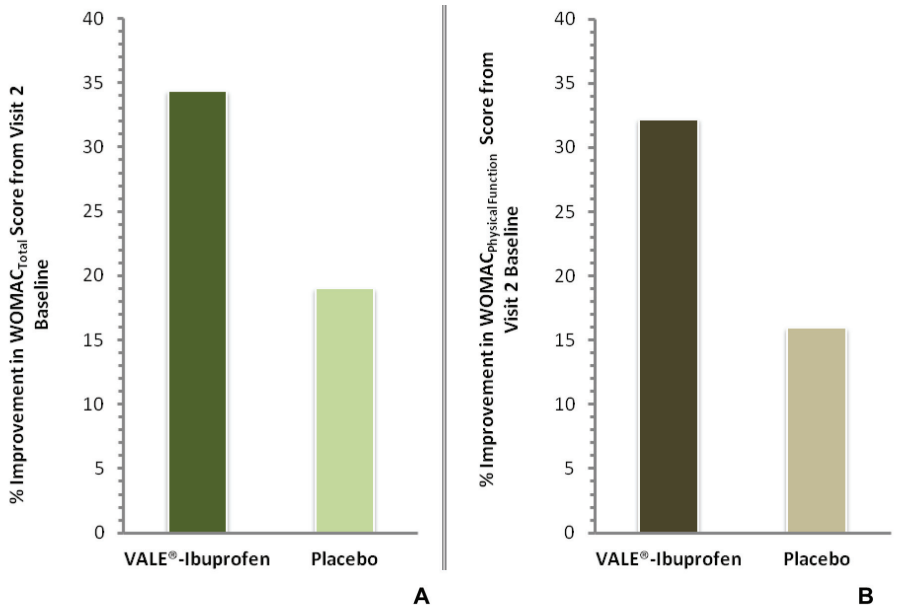


Fig. 4. *VALE-ibuprofen phase 2 clinical study.*
Panel A: Improvement in knee pain from Clinic Visit 2 to Clinic Visit 4 based on $WOMAC_{Total\ Score}$. VALE-Ibuprofen: N = 33; Placebo: N = 31. VALE-Ibuprofen has a 117.8% superior response compared to placebo, $P = 0.0283$. **Panel B:** Improvement in knee pain from Clinic Visit 2 to Clinic Visit 4 based on $WOMAC_{Physical\ Function}$. VALE-ibuprofen: N = 33; placebo: N = 31. VALE-ibuprofen has a 133% superior response compared to placebo, $P = 0.0201$.

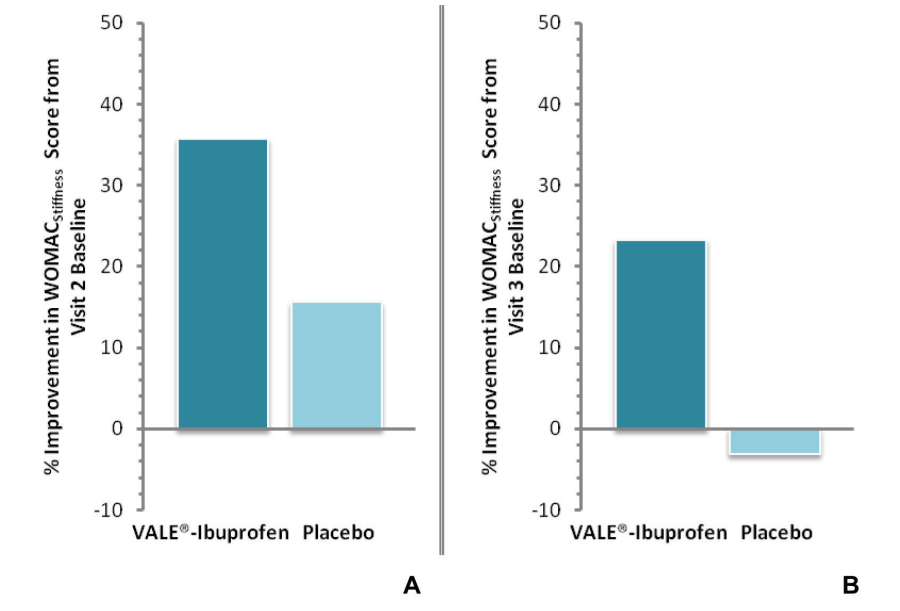


Fig. 5. *VALE-ibuprofen phase 2 clinical study: Improvement in knee pain based on* $WOMAC_{Stiffness}$.
Panel A: Improvement of $WOMAC_{Stiffness}$ score over placebo from Clinic Visit 2 to Clinic Visit 4. VALE-ibuprofen: N = 33; placebo: N = 31. VALE-ibuprofen is marginally superior compared to placebo, $P = 0.1103$. **Panel B:** Improvement of VALE-ibuprofen $WOMAC_{Stiffness}$ score over placebo from Clinic Visit 3 to Clinic Visit 4. VALE-ibuprofen: N = 33; placebo: N = 31. VALE-ibuprofen has as significantly superior response compared to placebo, with a statistical significance: $P = 0.0201$.

Table 3. *Descriptive statistics of observed clinical endpoint values by treatment group and clinic visit.*

	Ibuprofen Group Mean ± SD (n)		Placebo Group Mean ± SD (n)		*P* value (Ibuprofen v. placebo)
VAS REST					
Visit 1 (Day 0)	5.363 ± 1.091 (23)		5.264 ± 1.157 (18)		
Visit 2 (Baseline)	5.211 ± 1.582 (33)		4.789 ± 1.365 (31)		
Visit 3 (One week)	3.661 ± 2.438 (33)		3.331 ± 2.123 (31)		
Visit 4 (Two weeks)	2.758 ± 2.220 (33)		3.261 ± 2.367 (31)		
Changes from baseline (week one)	-1.550 ± 2.369 (33)	(*P* < 0.0001)	-1.458 ± 1.539 (31)	(*P* = 0.0002)	0.8556*
Changes from baseline (week two)	-2.453 ± 1.643 (33)	(*P* < 0.0001)	-1.527 ± 1.810 (31)	(*P* = 0.0001)	0.0785**
Changes from week one to week two	-0.903 ± 1.809 (33)	(*P* = 0.0026)	-0.069 ± 1.471 (31)	(*P* = 0.8161)	0.0482***
VAS MOTION					
Visit 1 (Day 0)	6.491 ± 1.432 (23)		6.144 ± 1.814 (18)		
Visit 2 (Baseline)	6.359 ± 1.923 (33)		6.118 ± 1.871 (31)		
Visit 3 (One week)	4.356 ± 2.505 (33)		4.553 ± 2.676 (31)		
Visit 4 (Two weeks)	3.791 ± 2.587 (33)		4.076 ± 2.615 (31)		
Changes from baseline (week one)	-2.003 ± 2.398 (33)	(*P* < 0.0001)	-1.565 ± 1.863 (31)	(*P* < 0.0001)	0.4191*
Changes from baseline (week two)	-2.568 ± 2.261 (33)	(*P* < 0.0001)	-2.042 ± 1.953 (31)	(*P* = 0.0001)	0.3243*
Changes from week one to week two	-0.565 ± 1.391 (33)	(*P* = 0.0206)	-0.477 ± 1.338 (31)	(*P* = 0.0562)	0.7982*
VAS WEIGHT					
Visit 1 (Day 0)	6.683 ± 1.885 (23)		6.224 ± 1.881 (17)		
Visit 2 (Baseline)	6.661 ± 2.029 (33)		6.506 ± 1.871 (31)		
Visit 3 (One week)	4.833 ± 2.814 (33)		4.740 ± 2.645 (31)		
Visit 4 (Two weeks)	3.911 ± 2.852 (33)		4.411 ± 2.794 (31)		
Changes from baseline (week one)	-1.827 ± 2.208 (33)	(*P* < 0.0001)	-1.766 ± 1.052 (31)	(*P* < 0.0001)	0.9071*
Changes from baseline (week two)	-2.750 ± 2.546 (33)	(*P* < 0.0001)	-2.095 ± 2.004 (31)	(*P* < 0.0001)	0.2594*
Changes from week one to week two	-0.923 ± 1.663 (33)	(*P* = 0.0012)	-0.329 ± 1.454 (31)	(*P* = 0.2463)	0.1345*
WOMAC PAIN					
Visit 1					
Visit 2 (Baseline)	4.925 ± 1.737 (32)		4.226 ± 1.508 (31)		
Visit 3 (One week)	3.570 ± 2.219 (33)		3.368 ± 1.829 (31)		
Visit 4 (Two weeks)	2.903 ± 2.206 (33)		3.090 ± 1.999 (31)		
Changes from baseline (week one)	-1.250 ± 1.863 (32)	(*P* < 0.0001)	-0.858 ± 1.393 (31)	(*P* = 0.0051)	0.3476*
Changes from baseline (week two)	-1.931 ± 1.904 (32)	(*P* < 0.0001	-1.135 ± 1.643 (31)	(*P* = 0.0007)	0.0811**
Changes from week one to week two	-0.667 ± 0.983 (33)	(*P* = 0.0020)	-0.277 ± 1.366 (31)	(*P* = 0.1968)	0.1935*

The difference between ibuprofen and placebo treated groups is: *= not significant; **trend towards significant; ***highly significant.

Table 3. (cont) *Descriptive statistics of observed clinical endpoint values by treatment group and clinic visit.*

	Ibuprofen Group Mean ± SD (n)		Placebo Group Mean ± SD (n)		*P* value (Ibuprofen v. placebo)
WOMAC PHYSICAL FUNCTIONING					
Visit 1					
Visit 2 (Baseline)	4.366 ± 1.710 (30)		3.768 ± 1.684 (31)		
Visit 3 (One week)	3.437 ± 2.001 (33)		3.273 ± 1.747 (31)		
Visit 4 (Two weeks)	2.961 ± 2.166 (33)		3.165 ± 2.135 (31)		
Changes from baseline (week one)	-0.964 ± 1.328 (30)	($P < 0.0001$)	-0.495 ± 1.038 (31)	($P = 0.0239$)	0.1291*
Changes from baseline (week two)	-1.494 ± 1.518 (30)	($P < 0.0001$)	-0.603 ± 1.393 (31)	($P = 0.0246$)	0.0201***
Changes from week one to week two	-0.477 ± 0.830 (33)	($P = 0.0082$)	-0.108 ± 1.160 (31)	($P = 0.5509$)	0.1489*
WOMAC STIFFNESS					
Visit 1					
Visit 2 (Baseline)	4.333 ± 2.416 (33)		3.145 ± 2.022 (31)		
Visit 3 (One week)	3.636 ± 2.251 (33)		3.242 ± 2.209 (31)		
Visit 4 (Two weeks)	2.788 ± 2.264 (33)		0.097 ± 1.665 (31)		
Changes from baseline (week one)	-0.697 ± 2.084 (33)	($P = 0.0292$)	-0.790 ± 1.419 (31)	($P = .0170$)	0.8358*
Changes from baseline (week two)	-1.545 ± 2.269 (33)	($P < 0.0001$)	-0.694 ± 1.909 (31)	($P = 0.0710$)	0.1103**
Changes from week one to week two	-0.848 ± 1.503 (33)	($P = 0.0031$)	3.935 ± 2.024 (31)	($P = 0.7348$)	0.0201***
WOMAC TOTAL					
Visit 1					
Visit 2 (Baseline)	4.546 ± 1.574 (29)		3.878 ± 1.552 (31)		
Visit 3 (One week)	3.481 ± 2.009 (33)		3.311 ± 1.696 (31)		
Visit 4 (Two weeks)	2.939 ± 2.144 (33)		3.140 ± 2.059 (31)		
Changes from baseline (week one)	-0.994 ± 1.351 (29)	($P < 0.0001$)	-0.567 ± 1.021 (31)	($P = 0.0103$)	0.1706*
Changes from baseline (week two)	-1.599 ± 1.579 (29)	($P < 0.0001$)	-0.738 ± 1.385 (31)	($P = 0.0074$)	0.0283***
Changes from week one to week two	-0.542 ± 0.848 (33)	($P = 0.0037$)	-0.171 ± 1.195 (31)	($P = 0.3591$)	0.1555*

The difference between ibuprofen and placebo treated groups is: *= not significant; **trend towards significant; ***highly significant.
This table summarizes the VAS values for: pain at rest, pain while walking and pain while standing: (VAS_{Rest} VAS_{Motion} VAS_{Weight} (mean±SD) at Visits 1, 2, 3, and 4, respectively. The table also summarizes the results of the WOMAC questionnaires from clinic visits 2, 3, and 4 ($WOMAC_{Physical}$ function, $WOMAC_{Pain}$, $WOMAC_{Stiffness}$, $WOMAC_{Total}$) (mean±SD). The statistical significance was calculated according to Analysis of Variances statistical method (ANOVA). The number of patients in each group is provided in parentheses.

Discussion

The goal of the Phase 2 clinical trial was to evaluate the safety and the efficacy of the drug product VALE-ibuprofen and compare it to a placebo over a 14 day period with twice-a-day transdermal dosing of 200 mg of ibuprofen on a single knee. The trial was conducted with 75 moderate to severe OA patients; some of whom were previously not responsive to established treatments. Prior treatments employed for these patients included medications, such as Acemetacin, Acetylsalicylic acid, Asasantin (aspirin combinations), Betaxolol, Celecoxib, Chondroitin Sulfate, Colchicine, Diclofenac, Ibuprofen (oral), Paracetamol, Pregabalin, Tenoxicam, and Tramadol. The efficacy evaluation of the VALE-ibuprofen indicated superiority over the placebo treatment in all of

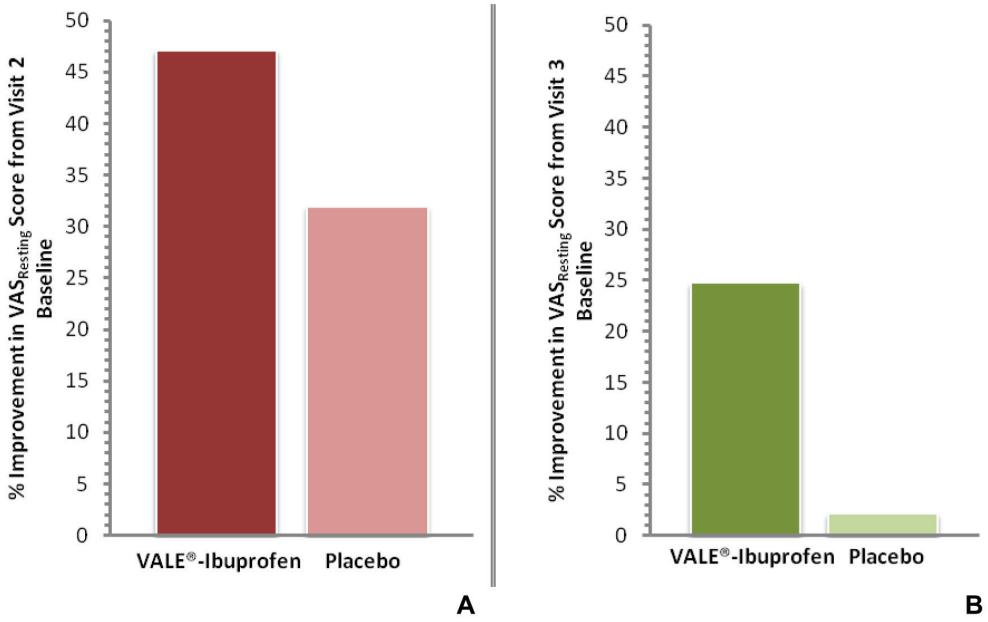


Fig. 6. *VALE-ibuprofen phase 2 clinical study.*
Panel A: Improvement in knee pain from Clinic Visit 2 to Clinic Visit 4 based on Visual Analog Scale$_{Pain}$. VALE-ibuprofen: N = 33; placebo: N = 31. VALE-ibuprofen has a 60.5% superior response compared to placebo, $P = 0.0785$. Panel B: VALE-ibuprofen Phase 2 Clinical Study: Improvement in knee pain from Clinic Visit 3 to Clinic Visit 4 based on Visual Analog Scale$_{Pain}$. VALE-ibuprofen: N = 33; placebo: N = 31. VALE-ibuprofen has a statistically significantly superior response compared to placebo, $P = 0.0482$.

Table 4. *Global Impression of Change. Combined patient's and investigator's assessment.*

	Ibuprofen		Placebo	
	Patient	**Investigator**	**Patient**	**Investigator**
Visit 2	No change (0.2289)	No change (0.5910)	No change (0.2289)	No change (0.5910)
Visit 3	Minimally improved (0.5491)	Minimally improved (0.6398)	Minimally improved (0.5491)	Minimally improved (0.6398)
Visit 4	Minimally improved (0.3622)	Minimally improved (0.4195)	Minimally improved (0.3622	Minimally improved (0.4195)

Significance of the Mann-Whitney's U-test is provided in parentheses.
At Visits 2, 3, and 4 both patients and investigators assessed the global impression of change by grading their condition with no change, minimally worse, much worse, minimally improved, and much improved characterization of their observation. The number of patients in each category were tabulated, the frequency of opinions calculated, and Mann-Whitney's U-test performed. The median results of the Mann-Whitney's U-test are depicted in the table and the 2-sided *P*-values are provided in parentheses.

the clinical assessment tests performed for the WOMAC tests as well as the VAS tests. Further, the efficacy of the ibuprofen group was also noted in each test with an intra-group analysis of the statistical significant improvement in the scores observed from the initial baseline (Clinic Visit 2) to the final Clinic Visit 4 (Week 2).

Evaluation of the clinical endpoints, such as the WOMAC$_{Total}$, WOMAC$_{Physical\ Functioning}$, WOMAC$_{Pain}$, WOMAC$_{Stiffness}$, and VAS$_{Resting}$ demonstrated significance of the VALE-ibuprofen over the placebo groups and also indicated that these beneficial improvements were either statistically significant ($P < 0.05$) or trending towards statistical significance ($P = 0.05 – 0.11$). In particular, analyses of the WOMAC tests indicated the superiority of the ibuprofen drug product over the 2 week clinical trial in comparison to those results from the placebo. However, as expected, placebo effect had an influence on the statistical significance in some of the tests.

Such effects are well-known in the literature (42-45), in particular, in the development of topically applied and transdermal NSAIDs delivery systems, with pain as a clinical endpoint. These effects are amplified with clinical trials using relatively small numbers of patients and with shorter length trials, as in this trial.

The safety evaluation of the repeated b.i.d. dosing over a 2-week period of either VALE-ibuprofen or placebo indicated essentially no significant negative safety concerns or adverse events with the use of these transdermal formulations.

Conclusions

The Phase 2 clinical trial for the evaluation of the VALE-ibuprofen indicates positive clinical results over the placebo for each of the clinical endpoints tested. There were significant improvements to the treatment of pain in patients with OA with an excellent safety profile, indicating the need for continued clinical development with a larger-size and longer-lasting clinical trial as the next step with VALE-ibuprofen.

Disclosures

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Funding for the clinical trial was entirely provided for by BioChemics, Inc., Danvers, Massachusetts. The funding organization did not participate in any phase of the data generation or data analyses; however, researchers at BioChemics did participate in the preparation of the manuscript. The principal investigator of the clinical research team had full access to the study data.

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