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# A double-blind, randomized, saline-controlled study of the efficacy and safety of co-administered intra-articular injections of Tr14 and Ze14 for treatment of painful osteoarthritis of the knee: The MOZArT trial

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ABSTRACT

*Introduction:* Osteoarthritis of the knee is a prevalent and painful condition increasing with age. The aim of this, the first well-controlled randomized controlled trial, was to evaluate the efficacy and safety of co-administered Traumeel[1] (Tr14) and Zeel[1] (Ze14) in patients with knee osteoarthritis (OA) experiencing moderate-to-severe pain.

*Methods:* This was a double-blind, multi-center, randomized, saline-controlled trial. Eligible patients meeting the American College of Rheumatology criteria for knee OA were randomized to 3, weekly intra-articular Tr14/Ze14 (n = 119) or saline (n = 113) injections. Primary efficacy endpoint was knee pain change from baseline (day 1) to end-of-study visit (day 99), as measured by the WOMAC Pain Subscore. Secondary endpoints included measures of WOMAC OA Index total and subscores, 50-foot walk test, and patient (PGA) and physician (PhGA) global assessments. Safety was assessed by vital signs, treated knee examinations, adverse events (AEs), and concomitant medications use. Effect sizes were calculated *post hoc* for consistency with published meta-analyses of standard-of-care treatments.

*Results:* 232 patients were randomized with no significant baseline differences. For the primary endpoint Tr14/Ze14 was significantly superior to saline (−32.0 vs. −25.5; p = 0.0383, 95% CI for difference: −12.40, −0.35). WOMAC and 50-foot walk pain measures showed statistically significant efficacy for pain relief over study days 15–99 (except Day 29). Safety profile showed no serious adverse events related to the treatment. PhGA indicated significant improvement for Tr14/Ze14 on Days 29, 71 and Day 85.

*Conclusion:* In this study, intra-articular Tr14/Ze14 provided significant pain relief compared to saline-control throughout the observation period. Treatment effect sizes were clinically relevant, since these were comparable to those reported for standard-of-care treatments. The safety profile was benign.

## 1. Introduction

Knee osteoarthritis (OA) is a very common condition with prevalence rising with age. Globally, 3.8% of the population is estimated to be affected, with prevalence peaking at around 50 years of age [1]. The Framingham Osteoarthritis Study demonstrated that OA increased with age, from 27% in patients younger than age 70, to 44% in patients age 80 years or older [2]. OA is a complex condition involving cartilage, subchondral bone and synovium, where degenerative and inflammatory processes are both prominent. Activation of matrix proteinases [3] and macrophages [4] seems to play a pivotal role. OA is commonly diagnosed with the appearance of typical symptoms of pain, stiffness after inactivity and reduced joint function, supported by imaging findings of changes in the joint [5].

The goals of OA treatment include alleviation of pain and improvement of functional status [6]. For pharmacological intervention

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[1] Traumeel and Zeel are registered trade names of Biologische Heilmittel Heel GmbH, Germany.

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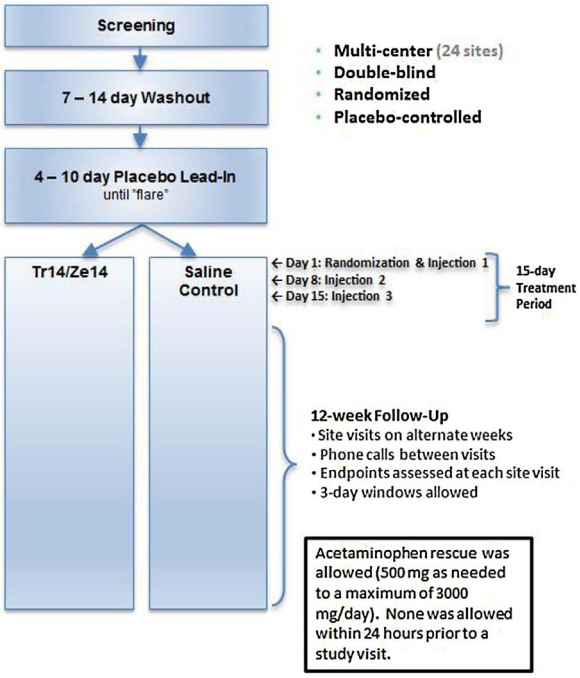


**Fig. 1.** Study Design.

the majority of current guidelines including OARSI[2] [7], ACR[3] [8], AAOS[4] [9], NICE[5] [10] and EULAR[6] [11] recommend the use of topical and oral nonsteroidal anti-inflammatory drugs (NSAIDs), tramadol, paracetamol, capsaicin and intra-articular (IA) injections of corticosteroids. Recommendations vary regarding use of symptomatic slow-acting drugs in osteoarthritis (SYSADOA), which are mainly represented by glucosamine sulfate, chondroitin sulfate and IA hyaluronic acid [12]. For example, the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) has published an algorithm recommendation, which includes SYSADOA already in the first step of the treatment [13]. Despite this broad range of therapeutic options, treating OA pain remains challenging due to side effects of existing treatment options such as NSAIDs, acetaminophen, and opiates, and due to co-morbidities, especially in an aging population [14].

In this study, IA injection of the combination of two medicinal products available on the market, Tr14 and Ze14, has been investigated in patients with moderate-to-severe pain associated with knee OA to assess its potential as a therapeutic approach in clinical practice. Tr14 and Ze14 have been available for over 20 years for treatment of musculoskeletal injuries (Tr14) and arthrosis/OA including rheumatic joint disease (Tr14/Ze14), and both have been utilized for the relief of pain and joint stiffness [15–18]. Tr14 and Ze14 are multicomponent homeopathic medicinal products. Each contains a total of 14 ingredients (i.e., there are 28 ingredients when combined). Components are of plant-origin, mineral-origin and other organic-origin, diluted to so-called “low potencies” since they contain measurable molecular concentrations of potential active agents [19]. The exact mechanisms of action are not known, but a number of studies suggest anti-inflammatory [20,21], antioxidative [22,23], and chondroprotective properties [24]. Deep sequencing transcriptome analysis in a wound healing model demonstrated expression changes induced by Tr14 in inflammation signaling genes, cell stress and damage markers, microRNAs, cell mobility indicators, extracellular matrix components, keratins, collagens and several previously uncharacterized genes [25], while other preliminary findings indicated potential inhibition of metalloproteinase-13 [26]. This research provides some insights on potential applicability of these two products in combination in OA, but no clinical studies have been performed to date.

Published clinical research on Tr14 focused on acute musculoskeletal injuries [18,27–29], and on chronic joint conditions in the case of Ze14 [17,30,31]. The current trial was conducted as a result of practicing rheumatologists in the US reporting anecdotal success to the manufacturer with IA coadministration of the Tr14/Ze14 (once-weekly for 3 weeks) for treatment of knee OA. It was observed that the pain reduction was already reduced after the 3rd injection, and patients were reporting good outcomes [32]. This prompted the respective rheumatologists to request modern data to support evidence-based prescribing. The current study tested this hypothesis using high-quality methodologies to determine if efficacy and safety data support routine use.

## 2. Methods

### 2.1. Ethics statement

The study was conducted in accordance with the principles of Declaration of Helsinki and good clinical practice guidelines and received institutional review board (IRB) approvals from each site via a central IRB (Quorum Review IRB, www.QuorumReview.com, Review File # 28156). Written informed consent was obtained from all participants involved in the study. None can be identified via this manuscript. All are fully anonymized. All mandatory laboratory health and safety procedures were in compliance. The trial was registered at the US National Institutes of Health (ClinicalTrials.gov): # NCT01887678.

### 2.2. Trial design

This was a prospective, multi-center, parallel-group, two-arm, double-blind, randomized, saline-controlled study conducted in the United States at 24 recruiting sites (Fig. 1).

Permitted variation around the total 16-week study period was: 7- to 14-day washout from prior treatments, 3 to 10 days lead-in eligibility period to observe knee OA flare, 2-weeks dosing, and 12-weeks follow-up. Study visits were at screening/start of wash-out period, start of lead-in period, baseline/randomization/first dose (Day 1), second dose (Day 8), third dose (Day 15), site visits on alternate weeks for 12 weeks, with phone calls to patients in-between visits (every-other-week).

### 2.3. Participants

232 patients were randomized, 45–80 years old, with knee OA according to the American College of Rheumatology criteria, with Kellgren-Lawrence Grade 2–3 radiographic criteria at screening [33]. Additional screening inclusion criteria were: currently taking an NSAID or acetaminophen regularly (4-7 days/week) over last 2 weeks with pain amelioration; 50-foot walk test pain score of $> 40$ mm on a 100 mm VAS in the target knee; and pain in the non-target (contralateral) knee $\leq 30$ mm on a 100 mm VAS on a 50-foot walk test. For randomization, primary complaint was pain following an unassisted 50-foot walk with: moderate-to-severe pain score in the target knee as demonstrated by 40–90 mm recorded on a 100 mm VAS scale; 20 mm increase in pain from screening visit pain score (“flare”); and pain in the non-target (contralateral) knee $\leq 30$ mm on a 100 mm VAS.

Major exclusion criteria were: known hypersensitivity or allergy to components of Tr14, Ze14 or acetaminophen; body mass index (BMI) $> 38$ kg/m$^2$; clinical symptoms of meniscal instability or significant valgus/varus requiring corrective osteotomy; any major injury or surgery to target knee in prior 12 months; any musculoskeletal comorbidities; skin disorder to area surrounding knee within previous 6

[2] Osteoarthritis Research Society International.
[3] American College of Rheumatology.
[4] American Academy of Orthopaedic Surgeons.
[5] National Institute for Health and Care Excellence.
[6] European League Against Rheumatism.

months; cancer treatment within previous 2 years, excluding skin carcinoma; viscosupplementation in target knee within prior 6 months; systemic corticosteroid or IA injection within prior 3 months; oral hyaluronic acid products, and/or oral glucosamine and/or chondroitin sulphate and/or diacerein within one month; opioids within 90 days; therapy with autologous stem cells or anticoagulants; or concomitant diseases that could affect knee-pain evaluation.

Data were collected at 24 experienced investigational sites from May 2013 to January 2014. Investigators were selected based on their medical credentials, prior experience conducting clinical trials, and prior OA clinical trial experience. Sites were selected based on having appropriately qualified site staff, including clinical nurses and study coordinators, as well as facilities appropriate to conduct the study per protocol requirements. Also confirmed were the adequacy of the investigational product (IP) storage, and assurance that there was appropriately designated unblinded staff to manage the proper handling and preparation of the IP. A site qualification visit was performed, and the investigators and their facilities were reviewed and approved by the IRB that had oversight for the study and patient safety.

The investigators and study staff were trained regarding the study protocol, data requirements and Good Clinical Practices at a dedicated investigators' meeting. Concordance training was conducted specific to the grading of roentgenograms to ensure proper Kellgren-Lawrence scoring to qualify patients for participation. Each investigator was required to demonstrate their ability to appropriately score roentgenograms prior to receiving approval to participate in the study. To further ensure consistency in identification of suitable patients and proper data collection, an on-site study initiation visit was conducted by a study- trained Clinical Research Associate (CRA) assigned to the investigative site. All sites were visited by a CRA throughout the study to ensure that enrolled patients were qualified based on the standard inclusion and exclusion criteria and that they appropriately consented, and that data collection was compliant with the protocol and training. The CRA was obligated to re-train the staff to ensure that the study was conducted in a consistent manner across all sites.

### 2.4. Interventions

Placebo oral tablets matching 500 mg naproxen were manufactured and packed in blister cards of 10 doses by Sharp Clinical Services, Inc., Phoenixville, PA. The injectable Tr14 and Ze14 solutions were manufactured and bottled in 2.2 mL and 2.0 mL glass ampoules, hydrolytic class I respectively by Biologische Heilmittel Heel GmbH, Germany, according to GMP standards. The study medication was packaged, shipped and labelled by Heel Inc., Albuquerque, NM, USA. The full description of ingredients and package labeling is available at the fda.gov website [34,35]. Each 10.0 mL vial of the saline-control contained: 0.9% sodium chloride preservative-free for injection. A total of 3 doses were injected into the target knee, one each at visits 3, 4 and 5 (Days 1, 8 and 15). Injection volume was 4.2 mL for both active study medication (2.2 mL Tr14 plus 2.0 mL Ze14) and saline-control. The injection procedure was identical for both treatments, standardized through concordance training (i.e., investigators were peer-educated regarding study diagnostic and treatment conventions). IA injection was performed per usual standard technique: precise, anatomical localization into joint cavity, and lateral or medial approach with patients either sitting or lying down, anterior approach was disallowed. For each patient, the same anatomic injection site and posture was used for all three injections. An anesthetic spray (i.e., ethyl chloride) was applied topically around the injection site.

Arthrocentesis was performed prior to injection and the amount of fluid removed was recorded. The control group experienced both arthrocentesis and saline injection. Consistent with knee OA (unlike rheumatoid arthritis), only minimal arthrocentesis fluid was generally expected and observed. Either a 25 gauge or 22 gauge 1.5 inch needle was used; needle size was consistent for each patient. If the fluid was cloudy, the patient was excluded and followed for possible infection. Otherwise, the needle remained in the joint space; the syringe was removed and replaced by a pre-filled syringe containing the assigned treatment. Patients were allowed to continue all routine daily-living activities after injection, but were advised not to overuse the treated knee for the first 48 h.

Each patient received acetaminophen 500 mg tablets to use as rescue medication, and diary-recorded dosing not to exceed 3 g/day (and not more than 4 consecutive days or 12 g in a 7-day period).

### 2.5. Outcomes

Methods of assessment included WOMAC[7] OA Index [36], 50-foot walk test and global rating of disease by patient and physician. Both WOMAC and an unassisted 50-foot walk test on a flat surface were measured by 100 mm VAS, the global assessements were rated on an ordinal scale from 1 (very good) to 5 (very poor). The primary efficacy variable was knee-pain change from baseline (day 1, predose) to end-of-study visit (day 99), as measured by the WOMAC Pain Subscore (A). Secondary variables included WOMAC pain subscore at each follow-up visit, WOMAC stiffness subscore, WOMAC physical function subscore, change in total WOMAC score, change in pain immediately following a 50-foot walk, change in time to walk (50-foot walk test), time to and use of rescue medication (follow-up using a patient diary), and patient's/physician's global assessment (PGA/PhGA). All secondary endpoints were assessed at screening where appropriate (visit 1), treatment (visits 3–5) and follow-up (visits 7, 9, 11, 13, 15, and 17).

### 2.6. Sample size

The sample size was determined *a priori* to provide 80% power to detect a difference of 10 mm between the treatments with respect to change from baseline in WOMAC pain subscore, assuming a standard deviation of 25 mm. These values were used because they are consistent with a published, knee-OA, standard-of-care product study [37], and with IMMPACT[8] [38] and ESCEO[9] [39] working group consensus recommendations.

### 2.7. Randomization and blinding

Placebo tablets were supplied at start of the lead-in period with a diary for documentation of dosings. The informed consent document executed prior to the lead-in period advised patients that "Everybody in this study will receive placebo during some part of the study." Consistent with ordinary practice for an analgesic-study placebo lead-in period, patients were told that the tablets are an analgesic taken twice daily (morning and evening). Placebo response was defined as pain improvement of $\geq 1$ unit on a 5-point Likert scale on $\geq 3$ continuous days during the oral placebo lead-in period. Patients exhibiting efficacy on oral placebo were subsequently not randomized for treatment. Eligible patients were allocated to treatment groups in a 1:1 computer-generated (PROC PLAN SAS version 9.1.3) randomization stratified by study site by an independent statistician.

At each study site allocation concealment was implemented by a designated, unblinded staff member not otherwise involved in the study, who prepared the appropriate injection in a separate location inaccessible to other study personnel based on the randomization number assigned by an automated web-based system linked to the randomization schedule. The system's algorithm provided patient-treatment assignments only when immediately required; an enrolling physician did not have access to assignments, but only received a

[7] Western Ontario and McMaster Universities Osteoarthritis Index.
[8] Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials.
[9] The European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases.

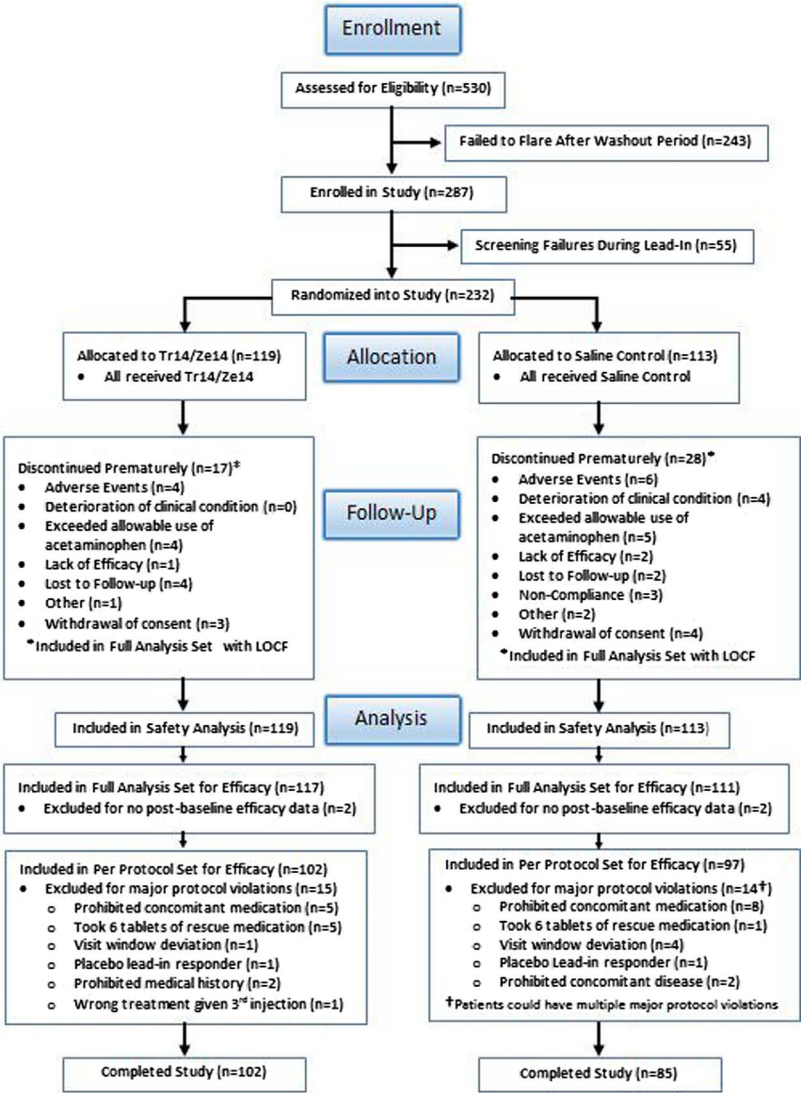


**Fig. 2.** Study Flow Diagram.

syringe with a clear, sterile solution ready for injection. Both injector/observing physician (evaluator) and patient were blinded to medication-preparation procedures. To assure mixing of Tr14 and Ze14 in a manner consistent with clinical practice, each site identified an unblinded staff member who drew the appropriate volumes of active and vehicle study injections and who subsequently provided this to the blinded injector for clinical use under blinded conditions. This unblinded worker was not permitted to have any other role in the study, and was not permitted to communicate in any way with patients/caregivers/injectors/evaluators about their study-related activities. Two clinical research organizations independently conducted activities to assure the integrity of the blind. Unblinded workers from Theorem Clinical Research GmbH and Company, KG, Hermann-Heinrich-Gossen-Str. 3, 50858 Köln/Germany conducted investigational medicinal product (IMP) accountability/integrity audits and communicated only with the clinical supply department of the sponsor charged with maintaining blinded conditions. Meanwhile, Accelovance Inc., 789 SW Federal Hwy, Suite 100, Stuart, FL, 34994 separately conducted site management activities/clinical monitoring and remained blinded along with the above listed study site personnel and patients/caregivers until database lock and subsequent release of the randomization list.

### 2.8. Data analysis

For each WOMAC scale, for the time to walk 50 feet, and for the resulting pain afterwards, analyses of covariance (ANCOVA) were applied to changes from baseline for each time point. These models included treatment as a qualitative factor, and baseline value as a covariate. In supplemental repeated measures analyses, each subject's changes from baselines were grouped across the visits and assumed to have a first-order autoregressive covariance; the fixed effects were the baseline value as a continuous covariate, and treatment, visit and treatment-by-visit interactions as categorical factors. For PGA and PhGA comparisons, Cochran-Mantel-Haenszel tests for ordered categorical responses stratified by baseline value were applied. Time to use of rescue medication was analyzed by Kaplan-Meier plots and log-rank tests. Since there were less than 10 patients per site on average, study site was not included as a stratification factor in the statistical analyses.

Treatments were compared at 2-sided 5% significance levels. No multiplicity adjustments were made because the primary endpoint compared only two treatments at a single time point, and the secondary endpoints were only supportive.

Primary efficacy analyses used a Full Analysis Set consisting of all patients who received at least one injection and for whom both a baseline value and at least one post-baseline value were recorded for the primary endpoint. Except for time to rescue medication, any missing data points were replaced by last observations carried forward (LOCF), since this has traditionally been used in regulatory submissions that were the basis of knee OA standard-of-care product approvals, and for treatments of chronic conditions in general. Sensitivity analyses

including repeated measures analysis with and without the LOCF were done on the WOMAC scales and the 50-foot walk pain and time. In a *post-hoc* assessment of whether Tr14/Ze14 pain effect sizes were consistent with standard-of-care treatments, Tr14/Ze14 Hedges' g* pain effect sizes [40] were calculated and compared to those reported in a historical meta-analyses of standard-of-care knee-OA treatment effect sizes [41].

Safety analysis set included all patients who received any study medication and were assessed by monitoring vital signs, target-knee physical examinations, and adverse events (AEs) during the study. In addition, regulatory authorities' Periodic Safety Update Reports and Development Safety Update Reports were compiled to assess consistency with the clinical trial results.

## 3. Results

Of the 30 qualified US investigational sites, 28 screened 530 patients; 287 patients were enrolled into the study in 24 centers; the remaining 243 did not flare following washout (Fig. 2). Fifty-five (55) enrolled patients did not qualify for randomization, of those 12 responded to oral placebos, 33 failed to meet eligibility criteria and 10 failed due to non-compliance, and were, therefore, lead-in screening failures (i.e., 232 patients were randomized at 24 sites). Overall, 187 patients completed the study, 102 in Tr14/Ze14 and 85 in saline groups. There were less than 10 completed subjects per center on average. Due to the limited number of subjects at each site, the data from all sites were pooled and site-differences could not be conclusively characterized.

Treatment groups had similar baseline demographic characteristics and clinical OA assessments (Table 1). Forty-five patients discontinued the study prematurely, 17 in Tr14/Ze14 and 28 in saline groups. Most frequent premature-discontinuation reasons were AEs; 4 patients in Tr14/Ze14 and 6 in saline groups; and a higher-than-allowed rescue-medication intake: 4 patients in Tr14/Ze14 and 5 in saline groups. There were higher discontinuation rates in the saline group.

All randomized patients formed the Safety Analysis Set with 119 patients in Tr14/Ze14 and 113 in saline groups. Fifteen of 117 patients in Tr14/Ze14 and 14 of 111 in saline groups had major protocol deviations (took prohibited concomitant medication, exceeded rescue medication limits, had excessive visit window deviation, responded to oral placebo lead-in, had prohibited concomitant disease or medical history, or were assigned a wrong treatment).

Table 2 and Fig. 3a show results for WOMAC Pain Subscale (A). The primary endpoint demonstrated that mean (SD) change in knee pain (mm) from baseline (Day 1, pre-dose) to end-of-study (Day 99) was −32.0 (26.88) in the Tr14/Ze14 and −25.5 (24.08) in the saline group; the difference was statistically significant, favoring Tr14/Ze14 ($p = 0.0383$, 95% CI for difference: −12.40, −0.35).

Furthermore, at all visits, mean pain decrease was higher in Tr14/Ze14 than in the saline group. Differences between treatment groups were statistically significant ($p < 0.05$) at all visits except Days 8 and 29. Results of the repeated measures analysis of WOMAC pain with and without the LOCF imputation were similar. The overall treatment effects were statistically significant for both analyses: $p = 0.0125$ with LOCF and $p = 0.0260$ without LOCF.

To assess the clinical relevance of these results, a *post hoc* analysis compared the treatments with respect to the proportions of patients for whom the improvements from baseline in WOMAC Pain Subscale met or exceeded a Minimum Clinically Important Improvement (MCII). The MCII was set at the highest level (36.6 mm) suggested for 100 mm VAS assessments of knee OA pain[42]. At the end of the study, 57 (48.7%) of the 117 Tr14/Ze14 patients met the criterion, compared to only 36 (32.4%) of the 111 patients who received only saline ($p = 0.0054$ by logistic regression).

Table 3 and Fig. 3b demonstrate that mean (SD) change in pain (VAS) from baseline to end-of-study following an unassisted 50-foot walk test improved significantly for Tr14/Ze14 compared to the saline-control, −43.1(26.37) mm versus −35.4 (24.72) mm, respectively (p-value = 0.0466). Results were consistent with the WOMAC Pain Subscale (Fig. 3a). Tr14/Ze14 was significantly superior to saline

**Table 1**
Baseline demographic and clinical characteristics.

| Demographic Data | Tr14/Ze14 Group (n = 119) | Saline-control Group (n = 113) | Total (n = 232) |
|---|---|---|---|
| Gender, n (%) | | | |
| Female | 76 (63.9) | 67 (59.3) | 143 (61.6) |
| Male | 43 (36.1) | 46 (40.7) | 89 (38.4) |
| Race, n (%) | | | |
| White | 91 (76.5) | 95 (84.1) | 186 (80.2) |
| Black Or African American | 24 (20.2) | 17 (15.0) | 41 (17.7) |
| Asian | 2 (1.7) | 1 (0.9) | 3 (1.3) |
| American Indian Or Alaska Native | 1 (0.8) | 0 | 1 (0.4) |
| Other | 1 (0.8) | 0 | 1 (0.4) |
| Age [years], Mean ± SD (median) | 60.7 ± 9.1 (60.0) | 59.7 ± 8.7 (59.0) | 60.2 ± 8.9 (59.5) |
| Height [cm] | 169.4 ± 9.4 (170.0) | 169.1 ± 10.9 (168.0) | 169.3 ± 10.1 (170.0) |
| Weight [kg] | 85.9 ± 16.3 (85.4) | 85.3 ± 18.1 (82.0) | 85.6 ± 17.2 (83.6) |
| BMI [kg/m$^2$] | 29.8 ± 4.4 (29.8) | 29.6 ± 4.2 (29.7) | 29.7 ± 4.3 (29.7) |
| Clinical Baseline Assessments, n (%) | | | |
| Site of OA target Knee | | | |
| Left | 58 (48.7) | 47 (41.6) | 105 (45.3) |
| Right | 61 (51.3) | 66 (58.4) | 127 (54.7) |
| Kellgren-Lawrence Grade | | | |
| Grade 2 | 67 (56.3) | 66 (58.4) | 133 (57.3) |
| Grade 3 | 52 (43.7) | 47 (41.6) | 99 (42.7) |
| Axis Deviation – Left Knee | | | |
| No | 106 (89.1) | 100 (88.5) | 206 (88.8) |
| Yes, thereof: | 13 (10.9) | 13 (11.5) | 26 (11.2) |
| Valgus | 5 (4.2) | 4 (3.5) | 9 (3.9) |
| Varus | 8 (6.7) | 9 (8.0) | 17 (7.3) |
| Axis deviation – Right knee | | | |
| No | 109 (91.6) | 102 (90.3) | 211 (90.9) |
| Yes, thereof: | 10 (8.4) | 11 (9.7) | 21 (9.1) |
| Valgus | 3 (2.5) | 3 (2.7) | 6 (2.6) |
| Varus | 7 (5.9) | 8 (7.1) | 15 (6.5) |

**Table 2**
Change in Knee Pain Measured by WOMAC Subscale A from Baseline (Day 1, Pre-dose) to Each Follow-Up Study Visit.

| Time Point | Tr14/Ze14 Group (n = 117) | Saline-control Group (n = 111) | Treatment Differences | | |
|---|---|---|---|---|---|
| | Mean (SD) | Mean (SD) | LS Estimate | 95% CI | p-value |
| Baseline | 53.2 (17.75) | 53.1 (18.70) | – | – | – |
| Visit 4/day 8 ± 1 | −15.8 (18.65) | −13.6 (19.11) | −2.15 | −6.89, 2.58 | 0.3715 |
| Visit 5/day 15 ± 1 | −24.4 (24.31) | −18.5 (20.02) | −5.87 | −11.15, −0.60 | 0.0293 |
| Visit 7/day 29 ± 3 | −26.8 (26.12) | −21.5 (21.62) | −5.24 | −10.88, 0.40 | 0.0686 |
| Visit 9/day 43 ± 3 | −29.7 (26.65) | −22.4 (22.33) | −7.25 | −13.08, −1.42 | 0.0150 |
| Visit 11/day 57 ± 3 | −30.4 (26.69) | −22.7 (23.37) | −7.56 | −13.54, −1.54 | 0.0134 |
| Visit 13/day 71 ± 3 | −31.0 (26.23) | −23.3 (24.38) | −7.60 | −13.52, −1.68 | 0.0121 |
| Visit 15/day 85 ± 3 | −31.1 (26.63) | −24.7 (24.57) | −6.32 | −12.28, −0.37 | 0.0370 |
| Visit 17/day 99 ± 3/End of Study[a] | −32.0 (26.88) | −25.5 (24.08) | −6.37 | −12.40, −0.35 | 0.0383 |

LS Estimates are the least square treatment differences, 95% CIs and 2-sided *p*-values based on ANCOVA with baseline (Visit 3) value as the covariate. Negative LS mean difference is in favor of Tr14/Ze14. Where appropriate, data were imputed with last observation carry forward (LOCF).
[a] Primary Efficacy Endpoint.

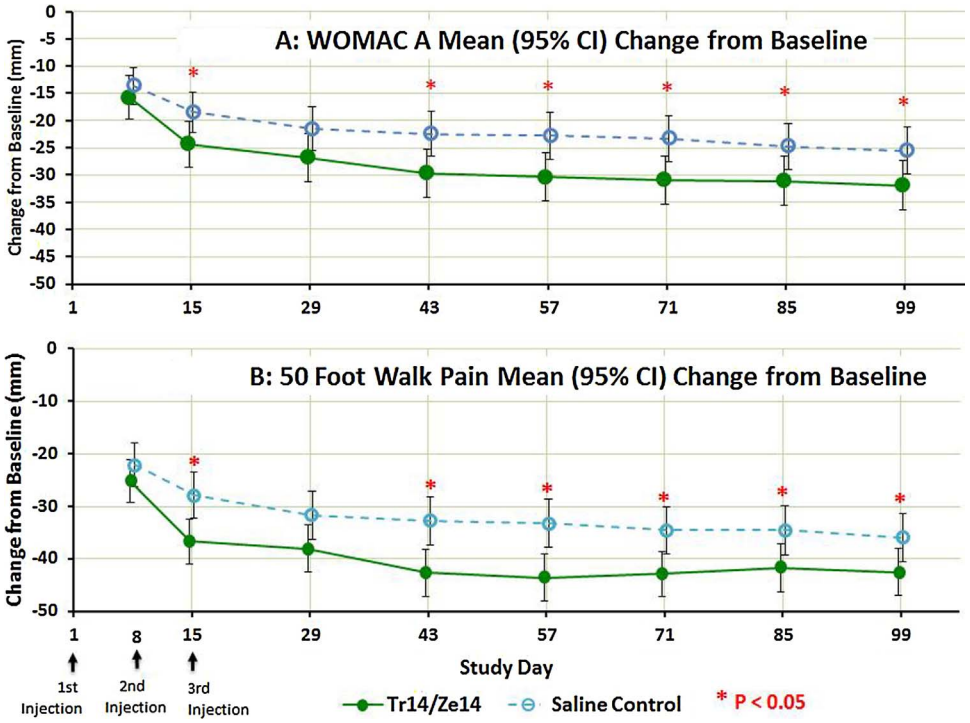


**Fig. 3.** Mean (95% CI) Change from Baseline in WOMAC A (Knee Pain Subscale, (A) and 50′ Walk Pain (B) versus Study Day (ITT Population, N = 228, LOCF).

($p < 0.05$) on all days post-Day 8 (time of 2nd of 3 weekly injections) except Day 29 ($p = 0.0501$). Change in time to walk 50 feet was not significantly different between active and saline treatments (data not shown).

Stiffness and physical function WOMAC Scores showed greater mean (SD) decreases from baseline to end-of-study visit in Tr14/Ze14 compared with saline: stiffness decreased from 57.7 (19.94) to 23.6 (25.38) mm and from 55.9 (19.27) to 27.7 (24.79) mm, respectively;

**Table 3**
Change in pain following an unassisted 50-feet walk (100 mm VAS) from Baseline to post-Baseline visits.

| Time Point | Tr14/Ze14 Group (n = 117) | Saline-control Group (n = 111) | Treatment Differences | | |
|---|---|---|---|---|---|
| | Mean (SD) | Mean (SD) | LS Estimate | 95% CI | p-value |
| Visit 4/day 8 ± 1 | −25.4 (22.65) | −21.8 (21.97) | −3.11 | −8.90, 2.68 | 0.2912 |
| Visit 5/day 15 ± 1 | −36.9 (25.23) | −27.5 (21.48) | −8.80 | −14.87, −2.72 | 0.0047 |
| Visit 7/day 29 ± 3 | −38.4 (25.66) | −31.3 (23.27) | −6.31 | −12.62, 0.00 | 0.0501 |
| Visit 9/day 43 ± 3 | −43.1 (25.13) | −32.3 (24.63) | −9.89 | −16.20, −3.58 | 0.0023 |
| Visit 11/day 57 ± 3 | −43.9 (24.75) | −32.8 (25.25) | −10.26 | −16.66, −3.86 | 0.0018 |
| Visit 13/day 71 ± 3 | −43.4 (25.46) | −33.9 (23.96) | −8.38 | −14.61, −2.15 | 0.0086 |
| Visit 15/day 85 ± 3 | −42.3 (27.37) | −33.9 (24.53) | −7.16 | −13.66, −0.66 | 0.0309 |
| Visit 17/day 99 ± 3 End of Study | −43.1 (26.37) | −35.4 (24.72) | −6.53 | −12.97, −0.10 | 0.0466 |

LS Estimates are the least square treatment differences, 95% CIs and 2-sided *p*-values based on ANCOVA with baseline (Visit 3) value as the covariate. Negative LS mean difference is in favor of Tr14/Ze14. Where appropriate, data were imputed with last observation carry forward (LOCF).

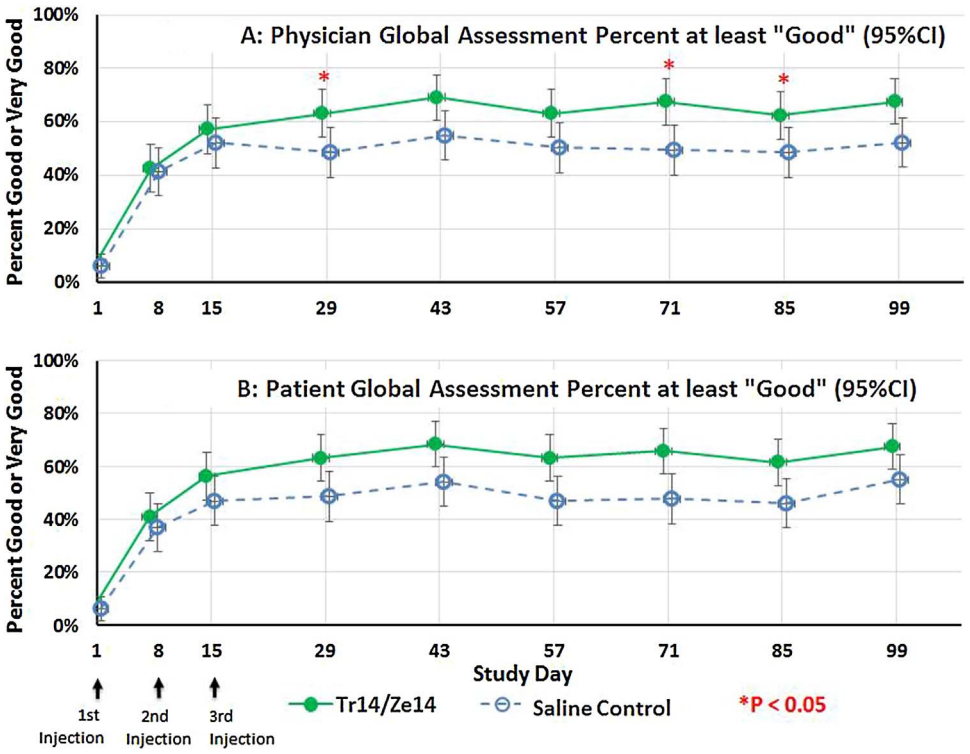


**Fig. 4.** Percent (95% CI) of Physician Global Assessments (A) and Patient Global Assessments (B) That Are "Good" or "Very Good" versus Study Day (ITT Population, N = 228, LOCF).

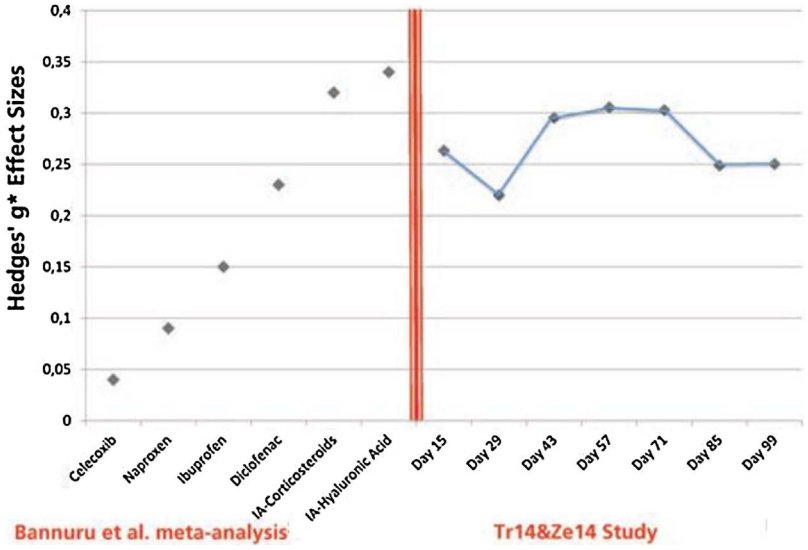


**Fig. 5.** Comparisons of Statistically Normalized (Hedges' g*) [40] Efficacy Effect Sizes for Tr14/Ze14 over Treatment Days 15–99 (from a Multi-center, Double-blinded Study) to PO Celecoxib, PO Naproxen, PO-Ibuprofen, PO-Diclofenac, IA Corticosteroids and IA Hyaluronates (from a Meta-analysis by Bannuru et al. [41]). All Effect Size Comparisons were to IA-Placebo.

physical function changed from 54.0 (18.82) to 22.9 (24.14) mm and from 54.2 (18.79) to 27.2 (24.43) mm, respectively. However, except for a significant difference in WOMAC B (stiffness) at Day 43 (mean changes from baseline −32.0 and −25.0 for Tr14/Ze14 and saline, respectively; 95% CI for difference: (−13.2, −0.8); *p*-value = 0.0276), no other comparisons were statistically significant. At all visits, the mean decreases in total WOMAC score from baseline to end-of-study were greater for Tr14/Ze14 versus saline; however, differences were only significant at Days 57 (mean changes from baseline −30.2 and −23.9 for Tr14/Ze14 and saline, respectively; 95% CI for difference: (−12.3, −0.2); *p*-value = 0.0430) and 71 (mean changes from baseline −30.6 and −24.6 for Tr14/Ze14 and saline, respectively; 95% CI for difference: (−11.9, −0.1); *p*-value = 0.0480).

At end-of-study, 68% of Tr14/Ze14 and 52% of saline patients had "good/very good" assessments (PhGA) compared to 6% at baseline (Fig. 4a). This was consistent with PGA, where 68% of Tr14/Ze14 and 55% of saline patients gave a "good" or "very good" evaluation, compared to 6% in both groups at baseline (Fig. 4b). Differences were statistically significant favoring Tr14/Ze14 for PhGA (not PGA) at Day 29 (p = 0.0062), Day 71 (p = 0.0238) and Day 85 (p = 0.0215).

Overall, 102 patients in Tr14/Ze14 and 87 in the saline-control groups reported taking rescue-medication, with a median number of 23.5 tablets in Tr14/Ze14 and 46.0 in the saline-control groups. After the first injection, median time to use of rescue medication [95% CI] was 2 [1,4] days for Tr14/Ze14 and 1 [1,2] days for saline; after the second injection, median time was 8 [2,17] days for Tr14/Ze14 and 1 [1,2] days for saline; and 5 [2,26] days for Tr14/Ze14 and 3 [2,8] days for saline after the third injection. No rescue medication was needed by 20 (17.1%) patients in Tr14/Ze14 and 13 (11.7%) in the saline-control groups.

The statistically normalized (Hedges' g*) efficacy effect sizes for Tr14/Ze14 compared to saline for the WOMAC OA Pain Subscale were

**Table 4**
Listing of SOCs and MedDRA Preferred Terms with at least 2 reported subjects N (%).

| | Tr14/Ze14 (N = 119) | Saline-control (N = 113) |
|---|---|---|
| Number of Patients with any TEAE N (%) | 64 (53.8) | 43 (38.1) |
| SOC or Preferred Term (indented) | | |
| Ear and labyrinth disorders | 2 (1.7) | 1 (0.9) |
| Eye disorders | 4 (3.4) | 1 (0.9) |
| Gastrointestinal disorders | 6 (5.0) | 3 (2.7) |
| Nausea | | 2 (1.8) |
| General disorders and administration site conditions | 11 (9.2) | 8 (7.1) |
| Pain | 5 (4.2) | |
| Injection site joint pain | 4 (3.4) | 1 (0.9) |
| Pyrexia | | 2 (1.8) |
| Infections and infestations | 12 (10.1) | 10 (8.8) |
| Nasopharyngitis | 4 (3.4) | 1 (0.9) |
| Urinary tract infection | 3 (2.5) | 1 (0.9) |
| Bronchitis | 2 (1.7) | |
| Upper respiratory tract infection | 1 (0.8) | 2 (1.8) |
| Injury, poisoning and procedural complications | 6 (5.0) | 6 (5.3) |
| Excoriation | 2 (1.7) | |
| Contusion | 1 (0.8) | 2 (1.8) |
| Investigations | 2 (1.7) | 5 (4.4) |
| Metabolism and nutrition disorders | 1 (0.8) | 3 (2.7) |
| Fluid retention | | 2 (1.8) |
| Musculoskeletal and connective tissue disorders | 26 (21.8) | 14 (12.4) |
| Arthralgia | 9 (7.6) | 8 (7.1) |
| Pain in extremity | 7 (5.9) | 2 (1.8) |
| Joint swelling | 3 (2.5) | 6 (5.3) |
| Back pain | 3 (2.5) | 1 (0.9) |
| Joint crepitation | 2 (1.7) | |
| Joint range of motion decreased | 2 (1.7) | |
| Muscle spasms | 2 (1.7) | |
| Nervous system disorders | 14 (11.8) | 8 (7.1) |
| Headache | 10 (8.4) | 3 (2.7) |
| Presyncope | 2 (1.7) | 1 (0.9) |
| Sciatica | 2 (1.7) | 1 (0.9) |
| Dizziness | | 2 (1.8) |
| Psychiatric disorders | | 2 (1.8) |
| Insomnia | | 2 (1.8) |
| Respiratory, thoracic and mediastinal disorders | 5 (4.2) | 3 (2.7) |
| Sinus congestion | 3 (2.5) | |
| Nasal congestion | 1 (0.8) | 2 (1.8) |
| Skin and subcutaneous tissue disorders | 4 (3.4) | 3 (2.7) |

Percentages are based on the number of patients in the safety analysis set within each treatment group. Percentages within system organ classes might add up to more than 100% if subjects had more than one event. Patients are only counted once in each system organ class or preferred term. Coding according to MedDRA Version 16.0.

0.26, 0.22, 0.30, 0.31, 0.30, 0.25 and 0.25 for Days 15, 29, 43, 57, 71, 85 and 99, respectively, indicating persistent efficacy over time. These values were comparable or superior to the statistically normalized effect sizes of independently reported standard-of-care IA and oral treatments versus IA placebo treatments published in the meta-analysis by Bannuru et al. (Fig. 5) [41].

Safety analysis included 232 randomized patients; 117 reported 231 adverse events (AEs) in total, consisting of 8 lead-in screening failure patients with 12 non-treatment-emergent AEs (non-TEAEs), 9 non-TEAEs in 7 patients and 210 treatment-emergent AEs (TEAEs) in 107 patients. Table 4 provides a listing of those System Organ Classes (SOCs) and Medical Dictionary for Regulatory Activities (Medra) Preferred terms with at least 2 reported subjects for either treatment.

No serious non-TEAEs were reported during lead-in. After randomization, 3/232 patients (1%) reported 4 serious TEAEs (both treatment arms). In Tr14/Ze14, 2/119 patients (2%) reported 3 events; for the saline-control, 1/113 patients (1%) reported 1 event. Reported Serious AEs (SAEs) were bradycardia and a hyperglycemic event in one Tr14/Ze14-treated patient, coffee-ground emesis in another Tr14/Ze14-treated patient, and a posterior circulation transient ischemic attack in a saline-treated patient. All SAEs occurred in a relevant time distance after the last injection, were considered to be not related to treatment, and resolved.

Treatment groups were similar with regard to AEs, particularly when considering that patients taking Tr14/Ze14 completed the study more often than saline-treated patients. Overall, 187 patients (80.6% of randomized patients) completed; Tr14/Ze14: 102 and saline: 85, 85.7% and 75.2% of the safety analysis set, respectfully. Overall, 45 (19.4% of the randomized patients) discontinued prematurely; Tr14/Ze14: 17 and saline: 28, 14.3% and 24.8% of the safety analysis set, respectively. The most frequent reasons for premature discontinuation were AEs in 10 (4.3%) randomized patients; Tr14/Ze14: 4 and saline: 6, 3.4% and 5.3% of the safety analysis set, respectively, and also a higher than allowed intake of rescue medication in 9 (3.9%) randomized patients; Tr14/Ze14: 4 and saline: 5, 3.4% and 4.4% of the safety analysis set, respectively. Forty-three (43), 7, 8 and 6 out of 119 patients with any TEAE in the Tr14/Ze14 group were considered unrelated, unlikely, possibly or probably related to treatment, respectively. This compares with 31, 4, 3, and 5 out of 113 patients in the saline group. Only single laboratory deviations and/or shifts were observed, with ranges within normal variability. No undue safety concerns were found.

To further explore safety, data were extracted from regulatory databases. Tr14 exposure was at least 117,333,284 ampoules or 2,257,043 patient-years with a cumulative 7 serious and 39 non-serious possibly-related adverse drug reactions (ADRs); Ze14 exposure was at least 30,168,795 ampoules or 580,169 patient-years with a cumulative 0 serious and 9 non-serious ADRs [43]. The serious ADR rate is well below the targeted 1:30,000 patient-years rate as recommended by the 2011 Consensus Guideline of the Osteoarthritis Research Society International for OA treatments being investigated in randomized clinical trials (RCTs) [44].

## 4. Discussion

This is the first prospective, saline-controlled, double-blind, randomized, clinical trial indicating that coadministered Tr14/Ze14 is an effective and safe IA treatment for moderate-to-severe pain in patients with knee OA. The study design is methodologically comparable to those used for modern clinical trials of standard-of-care treatments. It incorporated fully validated measures to assess treatment impact on pain associated with OA of the knee. In this study the primary endpoint, WOMAC knee pain change from the baseline, was statistically superior to saline. After an expected lag to maximum effect following first and second IA treatments (Day 3 and Day 8), there was significant improvement by Day 15 (third treatment). The delay-to-effect served as internal validation of the clinical model and efficacy endpoints. Improvements in pain were found at subsequent visits through to the end of the 3-month study, indicating a persistent therapeutic effect. At the end of the study, a statistically significantly higher proportion of Tr14/Ze14 patients met a criterion for minimum clinically important improvement. Consistent with pain alleviation as assessed by WOMAC, Tr14/Ze14 treatment improved patients' ability to walk without pain as measured by the 50-foot walk test. Therefore, two independent pain measures demonstrated an analgesic treatment effect after the 3rd injection, which is in agreement with the clinical practice observations reported by rheumatologists that initiated interest in conducting this study.

Median use of rescue medication was approximately double for saline- (46 doses) versus Tr14/Ze14-treated patients (24 doses). Patients could abstain from using rescue medication in the Tr14/Ze14 for a longer time compared to the saline group following the 2nd and 3rd IA injections. Arguably, this may indicate potential for a drug-sparing effect (e.g., to reduce the burden of concomitant oral NSAIDs needed for adequate treatment).

Notably, the treatment effects for pain seen in this study compare favorably to those in a meta-analysis of historical standard-of-care products. We calculated normalized (Hedges' g*) efficacy effect sizes [40] from the current study ranging from 0.22 to 0.31 after 2 of the 3 weekly doses were injected. In the most recently published meta-analysis from 129 studies/32,129 patients reported by Bannuru et al. [41], statistically normalized (Hedges' g*) effect sizes at 3 months compared to IA-placebo for standard-of-care products were: IA-hyaluronates 0.34, IA-corticosteroids 0.32, Per Os (PO)-diclofenac 0.23, PO-ibuprofen 0.15, PO-naproxen 0.09, PO-celecoxib 0.04, PO-acetaminophen (indeterminable). Our findings are consistent with those observed for IA-hyaluronates, IA-corticosteroids and oral non-steroidal anti-inflammatory drugs (NSAIDs) compared to IA-placebo.

One limitation of this study was that it was only statistically powered to discriminate pain, and was inadequate to assess function and/or stiffness, total WOMAC and PGAs. Also, the greater placebo response observed for IA-placebo compared to PO-placebo in the published meta-analysis may reflect physiological-injection effects [41] or differences in patients' expectations [45]. For knee OA, the route of administration has been shown to affect the magnitude of placebo effect [46–48]. Nonetheless, IA-saline is commonly categorized as an IA-placebo in clinical trials [41]. Since we excluded oral placebo responders from the current study, it might be argued that the procedure with arthrocentesis and saline injection may more appropriately be considered as a control. To obtain the most conservative comparisons possible between this study and literature values, treatment effects were compared to the knee OA placebo with the largest effect size, i.e, IA-placebo and not PO-placebo.

Independent meta-analyses were published prior to Bannuru et al. [41] that reported higher efficacy effect sizes for oral NSAIDs (0.29, 0.37), IA-corticosteroids (0.58), and IA-hyaluronates (0.60, 0.37–0.46) [7,49]. These effect sizes were estimated using standardized mean differences rather than the currently used Hedges' g*method; the latter approach includes a minor adjustment for sample sizes that is unlikely to account for the observed differences. It appears that such differences can be attributed to incremental availability of data over time. This is supported, for example, by changing OARSI recommendations between 2006 and 2010 for weight reduction and electromagnetic therapy [49].

In safety terms, AEs were similar in the Tr14/Ze14 and saline groups, particularly considering the greater persistence of exposure (lower discontinuation rates) for Tr14/Ze14 compared to saline. Consistent with safety data in the public domain, the safety profile was relatively benign with no signals of cardiovascular, gastrointestinal and/or other concerning risks.

The need for safe, effective and cost-effective treatments has been noted in current therapeutic guidelines [8–11]. Since approximately 47% of knee-OA sufferers use complementary medicines [50], evidence-based data in the form of randomized clinical trials and meta-analyses are in demand by the medical community; some are published but more are needed [50]. It is important to characterize the extent to which these treatments are safe and effective in managing symptoms and/or disease progression.

## 5. Conclusions

The results of this trial support consideration of Tr14/Ze14 as a therapeutic approach for patients with OA of the knee who fail to respond to standard-of-care pain treatments, or who are at risk due to comorbidities or adverse events. Further studies would be needed to determine if Tr14/Ze14 can slow and/or prevent progression of knee OA or mitigate drug toxicity (e.g., cardiovascular, renal, gastrointestinal) by reducing exposure to other treatments, such as NSAIDs. This may particularly apply to patients who can be treated with Tr14/Ze14 concurrently with standard-of-care products. In addition, studies will be needed to further define its place as a treatment for knee OA.

## Transparency section

### *Declaration of funding*

This study was financially supported by Biologische Heilmittel Heel GmbH, Germany (Heel). The company funded arms-length clinical trial deployment, data management/statistical analysis services, and clinical supply manufacture and accountability by employing two independent contract research service organizations to assure treatment-blinding. The company also provided technical support, including provision of scientific information and published literature regarding the tested products.

### *Declaration of financial/other relationships*

No financial arrangements with the authors have been made whereby the study outcome could affect compensation, that they can have any proprietary interest in the tested product, and that they can have an equity interest in the sponsor of the covered study. The authors served as consultants and planned/participated on expert panels to develop the protocol, write the statistical analysis plan, and write study reports. Dr. Lozada also consulted for Biologische Heilmittel Heel GmbH, Germany (Heel) by participating in scientific advisory board meetings and two symposia. The authors did not participate in study deployment, which was conducted at arms-length. The Contract Research Organization (CRO) for blinded site management and medical monitoring was Accelovance Inc., 789 SW Federal Hwy, Suite 100, Stuart, FL, 34994; the CRO for data management, unblinded monitoring of injectable IMP, medical writing and statistical analyses was Theorem Clinical Research GmbH & Co. KG, Hermann-Heinrich-Gossen-Str. 3, 50858 Köln/Germany. There were no other benefits or financial interests by the authors that could create a potential conflict of interest with regard to the work.

### *Author contributions*

All authors agree to be accountable for all aspects of the work.

Carlos J Lozada: conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article.

Eve del Rio: conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, administrative, technical and logistical support.

Donald P Reitberg: conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, administrative, technical and logistical support.

Robert A Smith: conception and design, analysis and interpretation of the data, statistical expertise, drafting of the article, critical revision of the article for important intellectual content, final approval of the article.

Charles B Kahn: conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article.

Roland W Moskowitz: conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article.

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