A Phase 2, Randomized, Double-Blind, Placebo-Controlled, Single-Dose Study of UBX0101 in Moderate to Severe, Painful Osteoarthritis of the Knee

Investigational Product	UBX0101
Protocol Number	UBX0101-MUS-201
Version Number	2.0
Version Date	17Mar2020
Amendment	1
IND Number	135033
Sponsor	UNITY Biotechnology, Inc.
Sponsor's Legal Representative	Ben Hsu, MD, PhD Vice President and Medical Director, General Medicine UNITY Biotechnology, Inc. 285 East Grand Ave South San Francisco, CA 94080

	Date
Original Protocol:	05 August 2019 Version 1.0
Amendment 1	17 March 2020 Version 2.0

SUMMARY OF CHANGES: AMENDMENT 1

The following summarizes the principal elements requiring that change be made to the protocol. Other minor modifications for administrative purposes have been made and are captured in the redlined document.

The Sponsor is implementing these changes based on precautionary measures taken in response to the COVID-19 outbreak.

The principal components of change to the study are described in the table below.

Area of change	Rationale
Table 1, Schedule of Events; Section 6.1.1: Remote visits and expanded visit windows	Remote visits for Visits 4–8 are added to provide an alternative to in-person clinic visits for investigative sites that are impacted by the COVID-19 outbreak. In light of the COVID-19 outbreak, visit windows are widened in order to accommodate more flexible scheduling of clinic visits. If a patient is unable to attend the clinic visit within the widened window, every effort should be made to conduct the visit assessment.

STATEMENT OF COMPLIANCE

The study will be conducted in compliance with this clinical study protocol, Good Clinical Practices (GCPs) as outlined by International Conference on Harmonisation (ICH) E6(R2), and all applicable local and national regulatory requirements. Enrollment at any clinical study site may not begin prior to that site receiving approval from the IRB or Ethics Committee of Record (ECR) for the protocol and all materials provided to potential patients. Screening at a site may not begin prior to approval from the IRB/IEC and the Sponsor.

Any amendments to the protocol or changes to the consent document will be approved by the IRB/IEC before implementation of that amendment. Reconsent of previously enrolled patients may be necessary, depending on the nature of the amendment.

All personnel involved in the conduct of this study have completed Human Subjects Protection and GCP training, as outlined by their governing institution.

SPONSOR'S APPROVAL

Title	A Phase 2, Randomized, Double-Blind, Placebo-Controlled, Single-Dose Study of UBX0101 in Moderate to Severe, Painful Osteoarthritis of the Knee
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Amendment	1

The design of this study, as outlined by this protocol, has been reviewed and approved by the Sponsor's responsible personnel as indicated in the signature table below.

Sponsor's Legal Representative:	**Title:**	**Signature:**	**Date:**
Ben Hsu, MD, PhD	Vice President and Medical Director, General Medicine		

INVESTIGATOR'S AGREEMENT

I have read the protocol, appendices, and accessory materials related to Study UBX0101-MUS-201 and agree to the following:

- To conduct this study as described by the protocol and any accessory materials
- To protect the rights, safety, and welfare of the patients under my care
- To provide oversight to all personnel to whom study activities have been delegated
- To control all Study Drug provided by the Sponsor and to maintain records of the disposition of those products
- To conduct the study in accordance with all applicable local and national regulations, the requirements of the ECR for my clinical site, and the GCPs as outlined by ICH E6(R2)
- To obtain approval for the protocol and all written materials provided to patients prior to initiating the study at my site
- To obtain informed consent or updated informed consent, in the event of new information or amendments, from all patients enrolled at my study site prior to initiating any study-specific procedures or administering Study Drug to those patients
- To maintain records of each patient's participation and all data required by the protocol

Name	**Title**	**Institution**
Signature		**Date**

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ACR	American College of Rheumatology
ADL	activities of daily living
AE	adverse event
AR	adverse reaction
BMI	body mass index
CBC	complete blood count
CFBL	change from baseline
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRA	clinical research associate
D	Day
DNA	deoxyribonucleic acid
ECG	electrocardiogram
ECM	extra-cellular matrix
ECR	Ethics Committee of Record
eCRF	electronic case report form
EDC	electronic data capture
GCP	Good Clinical Practice
IA	intra-articular
IA Inj	intra-articular injection
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IL	interleukin
IND	investigational new drug
IRB	Institutional Review Board
ITT	Intent-to-Treat
KL	Kellgren-Lawrence
LS	least squares
MDM2	mouse double minute-2
mITT	modified Intent-to-Treat
MMP	matrix metallopeptidase
MPsQ	Multidimensional Psychological Questionnaire
MRI	magnetic resonance imaging

Abbreviation	Definition
n	Number
N	total number
NCI CTCAE v4.03	National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03
NRS	numeric rating scale
NSAID	non-steroidal anti-inflammatory drug
OA	osteoarthritis
PD	pharmacodynamic(s)
PE	primary endpoint
PGA	Patient Global Assessment
PGIC	Patient Global Impression of Change
PK	pharmacokinetic(s)
PT	preferred term
R	randomization
Rb	retinoblastoma
RBC	red blood cell
SAE	serious adverse event
SAR	suspected adverse reaction
SASP	senescence-associated secretory phenotype
SE	standard error
SnC	senescent cell
SNRI	serotonin and norepinephrine reuptake inhibitor
SOC	system organ class
UBX0101	investigational product
ULN	upper limit of normal
US	United States
V	Visit
W	Week
WBC	white blood cell
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index
WOMAC-A	Western Ontario and McMaster Universities Osteoarthritis Index pain subscale
WOMAC-C	Western Ontario and McMaster Universities Osteoarthritis Index function subscale

1 SYNOPSIS

Title	A Phase 2, Randomized, Double-Blind, Placebo-Controlled, Single-Dose Study of UBX0101 in Moderate to Severe, Painful Osteoarthritis of the Knee
Phase	2
Study Design	This is a randomized, double-blind, placebo-controlled, single-dose, parallel-group study to assess the efficacy, safety, and tolerability of a single-dose intra-articular (IA) administration of UBX0101 in patients with moderate to severe painful knee osteoarthritis (OA).
Rationale	Despite the major potential for progressive disability associated with OA, all presently available treatments for the disease are symptomatic and focus on pain management, such as analgesics and anti-inflammatory agents, and have essentially no impact on its inevitable progression. UBX0101, a mouse double minute-2/p53 inhibitor, intervenes in the disease pathogenesis of OA by inducing apoptosis of senescent cells within tissues of the knee, reducing the production of pro-inflammatory, extra-cellular matrix-modifying, and regeneration-suppressing factors to halt, and potentially reverse, joint degeneration. Building on the favorable clinical responses, pharmacokinetic (PK) and safety results obtained during the Phase 1 study, this Phase 2 study is intended to assess the efficacy, safety, and tolerability of a single-dose IA administration of UBX0101 in symptomatic knee OA.
Target Population	This study will include patients ≥ 40 and ≤ 85 years of age with moderate to severe painful knee OA.
Number of Patients	Approximately: 180 Per treatment group: approximately 45
Length of Participation	The Screening period is 35 days and the treatment and follow-up period is 24 weeks, including a single dose of Study Drug administered on Day 0 and a final follow-up visit on Week 24.
Intervention	Group 1: Placebo IA at Week 0 Group 2: UBX0101 0.5 mg IA at Week 0 Group 3: UBX0101 2.0 mg IA at Week 0 Group 4: UBX0101 4.0 mg IA at Week 0 The four treatment groups will be enrolled concurrently.
Primary Objective and Primary Endpoint	Objective: Evaluate the effect of IA administration of UBX0101 on the change from baseline to Week 12 of pain in the target knee Endpoint: Change from baseline to Week 12 of the Western Ontario and McMaster Universities Osteoarthritis Index pain subscale (WOMAC-A) score in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo

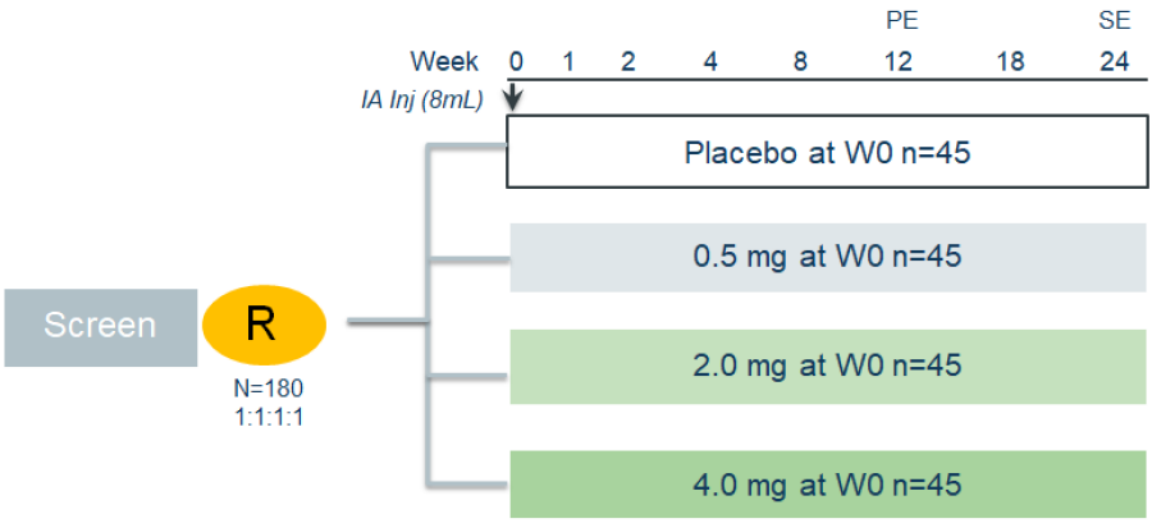
Secondary Objectives and Corresponding Endpoints	Objective: Evaluate the effect of IA administration of UBX0101 on the change from baseline to Week 12 in physical function Endpoint: Change from baseline to Week 12 of the Western Ontario and McMaster Universities Osteoarthritis Index function subscale (WOMAC-C) score in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo Objective: Evaluate the effect of IA administration of UBX0101 on the change from baseline to Week 12 in pain reported by patients daily over the 12-week study period Endpoint: Change from baseline to Week 12 of the weekly mean of the average daily pain intensity scores on the 11-point numeric rating scale (NRS) in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo Objective: Evaluate the effect of IA administration of UBX0101 on the duration of symptom improvement (pain and function) following IA administration of UBX0101 out to 24 weeks Endpoint: Change from baseline (over the entire 24-week period, including both the primary study period and the 12-week follow-up period) out to Week 24 for the WOMAC-A, NRS, and WOMAC-C scores in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo Objective: Evaluate the safety and tolerability of single-dose IA administration of UBX0101 Endpoint: Incidence of adverse events (AEs) as well as change from baseline through Weeks 12 and 24 in vital signs and selected laboratory safety parameters (as deemed clinically appropriate) in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo

Exploratory Objectives and Corresponding Endpoints	Objective: Explore the effects of single-dose IA administration of UBX0101 on the change from baseline in patient reported assessment of their osteoarthritis at the time of inquiry Endpoint: Change from baseline to Week 12 and to Week 24 in Patient Global Assessment score in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo Objective: Explore the effects of single-dose IA administration of UBX0101 on the change from baseline in patient reported impression of how their osteoarthritis has changed over the course of the study Endpoint: Change from baseline to Week 12 and to Week 24 in Patient Global Impression of Change score in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo Objective: Explore the effects of single-dose IA administration of UBX0101 on soluble biomarkers and imaging biomarkers of joint degeneration and repair Endpoints: • Change from baseline out to Week 24 in serum/plasma and urine biomarkers of synovium, cartilage, and bone degeneration • Change from baseline to Week 24 in magnetic resonance imaging-based markers of joint structure
Number of Sites	15–20
Study Duration	Estimated duration: 13 months
Safety Assessment Committee	A safety assessment committee will be established for adjudication of AEs or possible safety signals.

1.1 Study Schematic

The study schematic is presented in Figure 1.

Figure 1 Schematic of Study UBX0101-MUS-201



IA Inj=intra-articular injection; n=number; N=total number; PE=primary endpoint; R=randomization; SE= secondary endpoint; W=week.

1.2 Schedule of Events

The schedule of events is presented in Table 1.

Table 1 Schedule of Events for Study UBX0101-MUS-201

Activity[1]	**Screening D -35 to -1**	**V1 W0 D1**	**V2 W1 D8 (±5 days)**	**V3 W2 D15 (±5 days)**	**V4 W4 D29 (±10 days)** [17]	**V5 W8 D57 (±10 days)** [17]	**V6 W12 D85 (±10 days)** [17]	**V7 W18 D127 (±10 days)** [17]	**V8 W24 D169 (±10 days) Final Follow-up** [17]	**Unscheduled Visit/ Early Termination**[17]
Informed consent	X									
Eligibility criteria review	X	X								
Demographics	X									
Medical history	X	X								
Randomization		X								
Medication review[2]	X	X	X	X	X	X	X	X	X	X
Pregnancy test[3]	X	X							X	X
Physical examination[4]	X	X							X	X
Knee examination[5]	X	X	X				X		X	X
Vital signs[6]	X	X	X	X	X	X	X	X	X	X
ECG[7]		X								
Hematology and chemistry[8]	X	X	X		X		X		X	X
Drug screening urinalysis	X									
Knee X-ray	X								X	
Knee MRI[9]	X								X	
Study Drug administration[10]		X								
AE assessment		X	X	X	X	X	X	X	X	X
WOMAC	X	X	X	X	X	X	X	X	X	X
PGA[11]	X	X					X		X	X
PGIC[11]							X		X	X
NRS Daily Pain response review[12]	X	X	X	X	X	X	X	X	X	X
MPsQ[13]		X								

Activity[1]	**Screening D -35 to -1**	**V1 W0 D1**	**V2 W1 D8 (±5 days)**	**V3 W2 D15 (±5 days)**	**V4 W4 D29 (±10 days)** [17]	**V5 W8 D57 (±10 days)** [17]	**V6 W12 D85 (±10 days)** [17]	**V7 W18 D127 (±10 days)** [17]	**V8 W24 D169 (±10 days) Final Follow-up** [17]	**Unscheduled Visit/ Early Termination**[17]
PK blood sampling[14]		X								
Blood biomarker sampling[15]		X			X		X		X	X
Urine biomarker sampling[16]		X			X	X	X		X	X

AE=adverse event; CBC=complete blood count; D=day; ECG=electrocardiogram; MPsQ=Multidimensional Psychological Questionnaire; MRI=magnetic resonance imaging; NRS=numeric rating scale; PGA=Patient Global Assessment; PGIC=Patient Global Impression of Change; PK=pharmacokinetic; V=visit; W=week; WOMAC=Western Ontario and McMasters Universities Osteoarthritis Index.

[1] All procedures, with the exception of the knee examination and the PK blood sampling, should be performed predose. The knee examination should be performed predose and postdose.

[2] Medication review includes concomitant medications.

[3] Pregnancy test (only for females who are of childbearing potential): Serum pregnancy tests will be performed at a central laboratory at Screening and at Visit 8/ Week 24 or Early Termination. Urine pregnancy (dipstick) tests will be performed predose on the dosing day (Week 0).

[4] Physical examination includes assessments of the skin, head and neck, lungs, heart, abdomen, lymph nodes, extremities, and body weight and will be conducted at Screening and at and Final Follow-up (Visit 8/ Week 24) or Early Termination. Height will be measured at Screening only. After Day 1, symptom-directed physical examinations are required as clinically indicated.

[5] Knee examination: The target knee will be examined for erythema, palpable warmth, tenderness, swelling, effusion, crepitus, laxity, and range of motion.

[6] Vital signs include blood pressure, respiratory rate, pulse rate, and oral temperature. Patients will have a 5-minute rest in a sitting position before vital signs are assessed.

[7] 12-lead ECG evaluation will be performed predose for baseline reference.

[8] Hematology and chemistry: Screening tests in patients with diabetes should include hemoglobin A1c. If the Investigator deems appropriate, patients will be permitted to retest on clinical laboratory tests once while they are within the Screening window.

[9] Visit 8 MRI may be performed 14 days prior to Visit 8 to allow for scheduling flexibility.

[10] Dose will be administered using ultrasound guidance at Visit 1/Week 0/Day 1.

[11] All questionnaires will be conducted prior to vital signs, physical exams, ECGs, and blood draws. The wording and answer options of the PGA and PGIC question are referenced in Appendix 3.

[12] NRS daily pain score will be obtained for a minimum of 5 days during the Screening period to assess baseline pain for eligibility. Patients will complete an average daily pain question every day, from baseline through their last study visit, using an electronic diary. Clinical sites are to monitor ePRO responses at each visit to increase compliance. The NRS daily pain score is referenced in Appendix 2.

[13] MPsQ will be collected predose at Visit 1/Week 0/Day 1.

[14] PK blood sampling: On Day 1, blood will be drawn for plasma PK assessment at 2 hours postdose with a ±15-minute window.

[15] Blood biomarker samples to yield serum and plasma samples will be collected and processed according to the instructions in the Laboratory Manual.

[16] Urine biomarker samples will be collected and processed according to the instructions in the Laboratory Manual.

[17] In consideration of the COVID-19 outbreak, remote visits will be permitted at investigative sites, as appropriate, and should include collection of the WOMAC, adverse event assessment, concomitant medications review, NRS daily pain response review, PGA, and PGIC.

2 INTRODUCTION

UBX0101 is a new molecular entity, also described as Nutlin 3a (Vassilev et al. 2004), that is being investigated by UNITY Biotechnology, Inc. (the Sponsor) for the treatment of painful osteoarthritis (OA).

OA is the most common form of arthritis in which there is progressive loss of joint cartilage, changes to the subchondral bone, and inflammation of the synovium causing pain, stiffness, and functional disability impairing quality of life. Although OA can be found in any joint, it is most common in weight-bearing joints, with the knee being most affected, and in the hands (Litwic et al. 2013). Between the ages of 50 and 54 years, more than 13% of the population in the United States (US) was diagnosed with OA in 2016, and that rate rises to more than 31% for those over the age of 70 years (Institute for Health Metrics and Evaluation 2016). Finally, OA is associated with increased mortality (estimated odds ratio of 1.55), based on a meta-analysis of studies conducted by the OA Research Society International in a published white paper (OARSI White Paper Writing Group 2016).

2.1 Background

Despite the major potential for progressive disability associated with OA, all presently available treatments for the disease are symptomatic and focus on pain management, such as analgesics and anti-inflammatory agents (acetaminophen, non-steroidal anti-inflammatory drugs [NSAIDs], and cyclooxygenase-2 inhibitors) (Jüni et al. 2006), and have essentially no impact on its inevitable progression. Yet acetaminophen is poorly effective in OA, and the other products have safety and tolerability profiles that call into question the utility of these products in the broad population (da Costa et al. 2016). Beyond oral anti-inflammatory agents, the secondary treatments for OA involve intra-articular (IA) injection of glucocorticoids to reduce the inflammatory components of the disease or of hyaluronic acid to alter the joint mechanics to improve pain and functional use. Although glucocorticoids can reduce inflammation and pain, they do so for a limited period, may not be better than saline alone, and have been associated with thinning of cartilage (McAlindon et al. 2017). Furthermore, many patients have insignificant responses to glucocorticoids, and no measures are adequate predictors of response (Hirsch et al. 2013). Hyaluronic acid, while improving painful symptoms associated with OA, has a very limited objective clinical response and is only marginally better than saline alone (Johansen et al. 2016). Non-pharmacologic interventions, such as physical therapy, are often provided in the earliest stages of the disease (Bannuru et al. 2019).

Even with these interventions, many patients with the disease progress to the point of incapacitating pain or dysfunction. These patients are increasingly being considered as candidates for total knee arthroplasty. In 2011, over 700,000 knee arthroplasty procedures were conducted, and that rate is expected to climb as much as six-fold by 2030 (Ahmad et al. 2015; Kurtz et al. 2007). Although considered the only definitive treatment to alleviate OA, as many as 20% of patients undergoing arthroplasty are dissatisfied with the results of the procedure (Bourne et al. 2010). Overall, the socioeconomic burden of OA is high, with an estimated annual cost in the US of over $180 billion (Kotlarz et al. 2009).

The therapeutic interventions described previously, to greater and lesser extents, can partially lower the degree of inflammation present within the joint, thereby providing some degree of symptomatic relief. However, these interventions do not interfere with the underlying mechanisms that trigger the enhanced inflammatory response and therefore provide only limited duration of symptom relief without altering the course of the disease.

It is in this context of a disease with high rates of pain and suffering coupled with high socioeconomic costs that there is significant interest in the development of potential treatments, so called disease-modifying OA drugs, that would both significantly improve pain and function and fundamentally alter the course of the disease.

The Sponsor has identified a potentially important connection between fundamental biology associated with the process of aging and the development of OA. This biology refers to the cellular stress response known as senescence.

The principal feature of senescence, a regulated program in response to cellular stress, is to induce a cell to enter a permanent state of cell cycle arrest. The first observation that human cells do not divide indefinitely was proposed in 1961 by the seminal work of Leonard Hayflick (Hayflick and Moorhead 1961). Several important features of the senescence program have since been elucidated.

The connection between senescence and disease is highlighted by the fact that senescent cells (SnCs) acquire a bioactive secretome (Acosta et al. 2013; Coppé et al. 2008; Kuilman et al. 2008; Rodier et al. 2009). The development of this secretome, known as the senescence-associated secretory phenotype (SASP), is believed to be central to tissue abnormalities that arise when SnCs are present. While the actual components of the SASP can vary based on the stressors that are responsible for senescence induction or cell type of origin, it has been shown to be made up of more than 100 molecules that include those from the families of growth factors (e.g., vascular endothelial growth factor, transforming growth factor beta), inflammatory mediators (e.g., interleukin [IL]-1, IL-6) (Jeon et al. 2017), extra-cellular matrix (ECM)-altering enzymes (e.g., matrix metallopeptidase [MMP]-3 and MMP-13) (Jeon et al. 2017), and pro-inflammatory prostanoids (e.g., prostaglandin E2) (Martien et al. 2013). Thus, the inflammatory- and ECM-modifying milieu produced by SnCs are proposed to be the proximate cause of tissue pathology (Campisi and d'Adda di Fagagna 2007).

An additional aspect of senescence is that its induction can be caused by several different cellular stressors including oxidative or metabolic stress, deoxyribonucleic acid (DNA) damage, oncogene activation, or telomere shortening (Muñoz-Espín and Serrano 2014).

Two main pathways are responsible for the initiation and maintenance of senescent growth arrest: p53-p21-retinoblastoma (Rb) and p16Ink4a-Rb (van Deursen 2014). A key protein in the first of these pathways, p53, is a transcription factor activated after cellular stress. It regulates several downstream proteins, including p21, which inhibits cyclin dependent kinase-2, and is implicated in cell-cycle control, apoptosis, DNA repair, and senescence (Vogelstein et al. 2000; Vousden and Lu 2002). The latter pathway, involving activation of p16Ink4a, depends on signaling via cyclin dependent kinase-4 and -6 and, like p21, prevents inactivation of Rb, which results in the state of growth arrest (van Deursen 2014).

The fact that there is a strong overlap between mediators that exist in the OA inflammatory state and the SASP suggests, at the very least, an association between the disease and senescence. A stronger relationship between the two was highlighted by a study in rodents that demonstrated that removal of naturally occurring SnCs resulted in a greatly reduced degree of cartilage degeneration in the spine along with other marked phenotypes, including improved kidney function, reduced incidence of cataracts, and significantly lowered burden of atherosclerotic plaque along with a 30% improvement in lifespan (Baker et al. 2016; Childs et al. 2016).

Additional work has provided greater evidence for a cause-effect linkage between the accumulation of SnCs in the joint and the development and progression of OA. In animal

models of post-traumatic OA, induction of damage correlates with a rise in both the accumulation of SnCs (based on the upregulation of p16Ink4a) and SASP factors. Furthermore, deletion of these cells using a transgenic model that enables clearance of p16-positive cells or by treatment with UBX0101 attenuated the development of post-traumatic OA, reduced pain, and increased cartilage development. Additionally, cartilage tissue taken from human-donor knees, placed in a three-dimensional culture system, and treated with UBX0101 significantly reduced markers of senescence and upregulated production of both type II collagen and aggrecan, the two largest components of cartilage (Jeon et al. 2017).

A cross-sectional translational medicine study (Study UBX-OA-TNID-0001; ClinicalTrials.gov Identifier: NCT03100799) was recently conducted by the Sponsor to evaluate the relationship between various measures associated with OA and the extent of SnC accumulation in the knees of 30 patients with the disease. Four distinct associations with p16 were identified: with synovial fluid concentration of the cytokine IL-6; with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscale of patient-reported outcomes; with synovial thickness as determined by a contrast-enhanced magnetic resonance imaging (MRI)-based synovitis scoring system; and with the extent of OA as measured by the Kellgren-Lawrence (KL) grade (Data on file, UNITY Biotechnology).

Recently, the Sponsor completed a Phase 1, first-in-human study of a senolytic, UBX0101, in an OA study population (Section 2.1.3). The Phase 1 study yielded promising results in terms of clinical responses, pharmacokinetics (PK), and safety, which warrant further investigation of UBX0101 as a treatment for knee OA.

2.1.1 Target Indication and Population

This study will enroll patients ≥ 40 and ≤ 85 years of age with moderate to severe painful knee OA.

2.1.2 Description of UBX0101

UBX0101 is a mouse double minute-2 (MDM2)/p53 inhibitor intended to induce apoptosis in SnCs found in the joints of patients with OA. The transcription factor p53, as mentioned above, is a major stress response integrator (Bieging et al. 2014). In addition to being one initiator of the senescence program, another critical function of p53 is the activation of the apoptosis machinery (Khoo et al. 2014). The current model for which p53 leads to apoptosis resides in its ability to upregulate pro-apoptotic proteins above a critical level for which anti-apoptotic proteins can no longer counterbalance (Khoo et al. 2014). Cellular levels of p53 are primarily regulated via proteasomal degeneration after ubiquitination by MDM2, an E3 ubiquitin ligase (Wade et al. 2013). The human homolog is also referenced as MDM2 but has also been referred to as HDM2, RING finger protein, MGC5370, or ACTFS (HGNC Database). When a cell is under stress, post-translational modifications on p53 lead to breakage of its interaction with MDM2. By not being ubiquitinated, p53 then escapes proteasomal degeneration and accumulates (Bieging et al. 2014). Small molecules like UBX0101 bind to MDM2 causing a displacement of p53, thereby preventing its degeneration and leading to apoptosis of cancer cells (Khoo et al. 2014; Vassilev et al. 2004). Similarly, UBX0101 treatment leads to an accumulation of p53 and, consequently, induction of apoptosis in SnCs.

Based on the full range of results, the Sponsor is developing a therapy to intervene in the disease pathogenesis of OA by inducing apoptosis of SnCs within tissues of the knee, reducing the production of pro-inflammatory, ECM-modifying, and regeneration-suppressing factors to halt, and potentially reverse, joint degeneration (Pelletier et al. 2001).

2.1.2.1 Administration Regimen

UBX0101 0.5, 2.0, or 4.0 mg or placebo will be administered once via IA injection at Week 0. IA injection is administered using ultrasound guidance.

2.1.2.2 Justification for Dosing Strategy

The UBX0101 dose regimens planned for evaluation in this study were selected based on results from the Phase 1 Study UBX0101-OAR-101 (NCT03513016), which included a first-in-human, single-ascending dose evaluation (Part A) to establish an appropriate UBX0101 dose followed by a 6-week safety follow-up to collect additional safety and tolerability data.

In Part A of the Phase 1 study, single IA doses of 0.1, 0.2, 0.4, 1.0, 2.0, and 4.0 mg UBX0101 were studied. Following 12 weeks of data collection, doses of 1.0, 2.0, and 4.0 mg resulted in clinically significant improvements in both pain and physical function compared to placebo treatment. The findings are consistent with predictions from the UBX0101 pharmacokinetic (PK) model incorporating in vivo data and estimated human equivalent synovial concentrations that doses of 1.0 mg and higher administered via IA injection may produce sufficient exposure in the knee to induce apoptosis of SnCs in joint tissue.

Single IA doses of 0.5, 2.0, and 4.0 mg UBX0101 will be evaluated in the Phase 2 study. The highest single IA doses of 2.0 and 4.0 mg UBX0101 were informed by Phase 1 results, which showed a clear separation of the 2.0 and 4.0 mg doses from placebo on measures of pain and physical function over the 12-week study period. These will be repeated in the Phase 2 study to confirm the results observed over 12 weeks in the Phase 1 study in a larger population. The lowest dose chosen to establish the minimal- to no-effect level, 0.5 mg UBX0101, is supported by the findings at doses of 0.1, 0.2, and 0.4 mg UBX0101 in Phase 1, suggesting minimal to no difference in WOMAC pain subscale (WOMAC-A) versus placebo but mixed results when comparing over the 12-week study period using the numeric rating scale (NRS) measure of daily pain. In addition, in the Phase 2 study, all IA doses, including placebo, will be standardized to an 8 mL injection volume.

2.1.3 Supportive Clinical Data

The Phase 1 Study UBX0101-OAR-101 (NCT03513016) represents the clinical experience to date with UBX0101 administered by IA injection. The study population was adults age ≥ 40 to ≤ 85 years with moderate to severe, painful knee OA.

In Part A, the single ascending dose portion of the study evaluating six dose levels of UBX0101 (between 0.1 and 4 mg), 48 patients were randomly assigned to receive UBX0101 or placebo. Primary endpoints were safety and tolerability. Secondary and exploratory endpoints included plasma PK, synovitis as measured by MRI, pain (as measured by WOMAC-A and NRS), and measurement of SASP factors and disease-related biomarkers present in synovial fluid and plasma.

In Part B, 30 patients were to receive a single-dose IA administration of UBX0101 (4.0 mg) or placebo in a 2:1 randomization. Primary endpoints were safety and tolerability. Secondary and exploratory endpoints included changes in the levels of SASP factors and disease-related biomarkers present in synovial fluid and plasma as well as pain at 4 weeks only. Synovial fluid samples were obtained at baseline and 4 weeks post-treatment.

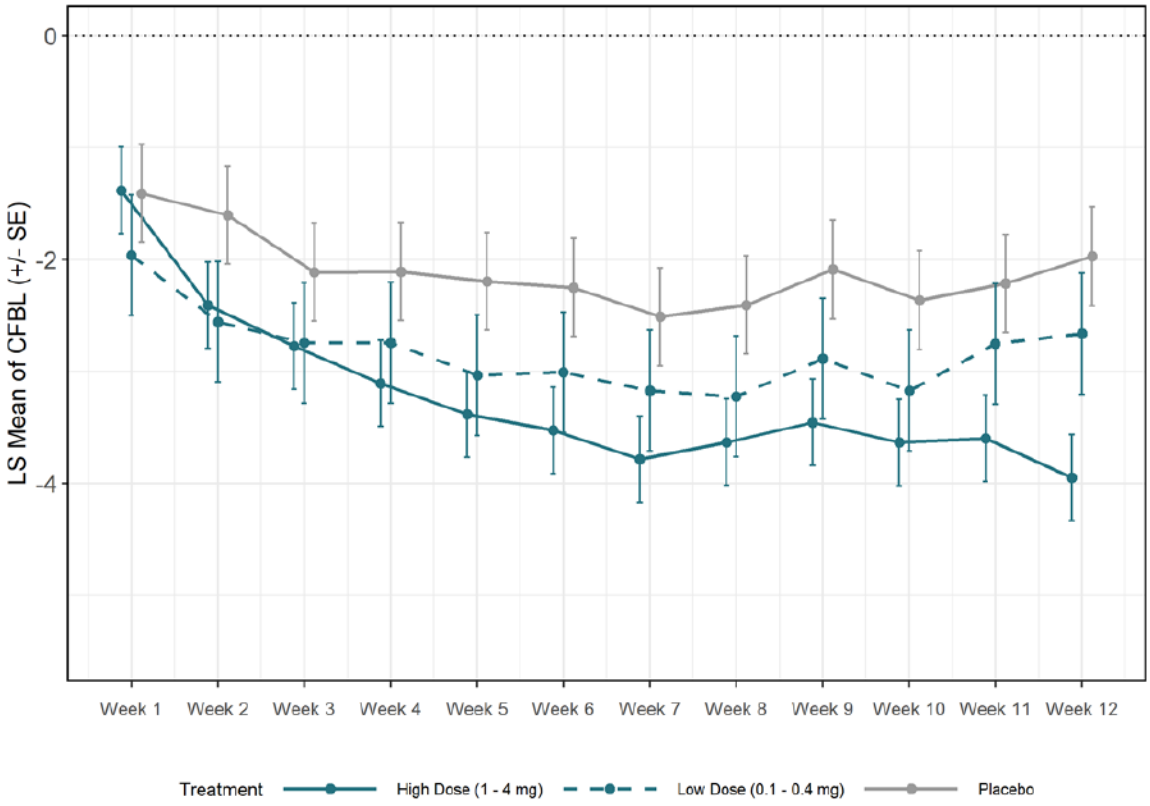
In the Phase 1 study, single doses of UBX0101 IA up to 4 mg administered to patients with painful knee OA were found to be safe and generally well tolerated. During the study, 65 adverse events (AEs) were reported by 36 out of 78 patients (56 AEs in 28 patients in Part A

and 9 AEs in 8 patients in Part B). Most AEs were rated as mild in intensity. No dose-dependent pattern of AEs was observed. There were no serious adverse events (SAEs) and no AEs that led to discontinuation.

In Part A, 7 AEs considered related to Study Drug were reported in 6 patients: procedural pain (placebo-treated patient), arthralgia (0.4 mg dose patient), muscle spasms (0.4 mg dose patient), OA (1.0 mg dose patient), procedural pain and synovitis (both in one 2.0 mg dose patient), and post-procedural swelling (4.0 mg dose patient). In Part B, 3 AEs considered related to Study Drug were reported in 2 patients: procedural pain and joint effusion (both in the same 4 mg dose patient) and procedural pain (4.0 mg dose patient). No clear dose dependence of causally related AEs was observed in this Phase 1 study.

In terms of clinical responses, patients treated with single IA injections of UBX0101 had clinically meaningful and dose-dependent improvements in knee pain symptoms as measured by the average daily pain NRS (0–10 point scale) and the WOMAC-A (Likert scale, 0–4) at 12 weeks compared to those who received placebo treatment. In a prospectively defined analysis in which the placebo (n = 14), low dose combined 0.1 through 0.4 mg UBX0101 (n = 16), and high dose combined 1 through 4 mg UBX0101 (n = 18) groups were compared, the least squares (LS) mean of the change from baseline (CFBL) in NRS at Week 12 was significantly greater for the high dose UBX0101 group (-3.95, $p < 0.01$) compared to the placebo (-1.96) and low dose groups (-2.66, $p = 0.42$ versus placebo). Especially for the UBX0101 4 mg highest dose group, LS mean reductions from baseline in the NRS of 4 points and greater up to Week 12 suggested a large magnitude UBX0101 treatment effect. Similarly, pain reduction as measured by WOMAC-A was clinically meaningful and greater in the high dose UBX0101 group compared to the placebo and the low dose groups. For the higher doses, the pain responses appeared to be durable through 12 weeks. The improvements in pain NRS over time in Part A are presented in Figure 2.

Figure 2 NRS Weekly Least Squares Mean Change From Baseline to Week 12



CFBL=change from baseline; LS=least squares; NRS = numeric rating scale; SE=standard error.

Clinically meaningful, dose-dependent improvements in physical function as measured by the Western Ontario and McMaster Universities Osteoarthritis Index function subscale (WOMAC-C) were observed and were greater in UBX0101-treated patients compared to those who received placebo. Evaluation of Patient Global Impression of Change (PGIC) following UBX0101 treatment demonstrated a higher proportion of patients being “much improved” or “very much improved” versus placebo at 12 weeks (placebo = 42.9%, low doses of UBX0101 [0.1, 0.2, and 0.4 mg] = 50.0%, high doses of UBX0101 [1.0, 2.0, and 4.0 mg] = 61.1%).

In Part B, the UBX0101 4.0 mg treatment group (n = 20) had a numerically greater mean reduction in WOMAC-A at 4 weeks compared to the placebo group (n = 10), but this was not statistically significant. The different magnitudes of treatment effect on WOMAC-A for the UBX0101 4.0 mg groups in Parts A and B may be due to factors including different study designs, study cohorts and investigative sites, frequency and timing of pain reporting, and the focus on arthrocentesis and lavage procedures to collect synovial fluid in Part B.

In Part B, 19 biomarkers were analyzed across 20 matched, paired synovial fluid samples. In approximately half the biomarkers measured in synovial fluid (treatment versus placebo) modulation was observed consistent with elimination of SnCs and potential improvement in the tissue environment. Changes were observed in matrix metalloproteases, tissue remodeling factors, and inflammatory cytokines.

In summary, single doses of UBX0101 up to 4.0 mg were safe and well-tolerated in patients with knee OA. Dose-dependent clinical responses in patient-reported measures of pain and function,

and UBX0101 treatment resulted in modulation of multiple biomarkers in the synovial fluid. Together, these results are encouraging, and suggest that a senolytic drug can at the same time address both symptoms of knee OA and the pathophysiology of structural progression.

2.1.4 Benefit:Risk Assessment

As described in Section 2.1, despite the major potential for progressive disability associated with OA, all presently available treatments for the disease are symptomatic and focus on pain management, such as analgesics and anti-inflammatory agents, and have essentially no impact on its inevitable progression. UBX0101, a MDM2/p53 inhibitor, intervenes in the disease pathogenesis of OA by inducing apoptosis of SnCs within tissues of the knee, thus reducing the production of pro-inflammatory, ECM-modifying, and regeneration-suppressing factors. As such, UBX0101 intervention in OA pathogenesis may ultimately halt or potentially reverse joint degeneration.

In the Phase 1, first-in-human study (Study UBX0101-OAR-101), single doses of 0.1 mg up to 4.0 mg UBX0101 administered as IA injections into the target knee joint were well tolerated in patients with OA up to and including a dose of 4.0 mg. There were neither SAEs nor AEs that led to study discontinuation. In the overall study population of 78 patients, 65 non-serious AEs were reported in 36 patients; no dose-dependent pattern of AEs was observed. The three highest doses of UBX0101 (1, 2, and 4 mg) resulted in clinically meaningful improvements in pain and physical function compared to placebo as measured by the 11-point NRS, WOMAC-A, and WOMAC-C. Additionally, the improvements in WOMAC-A and WOMAC-C had rapid onset within 1–2 weeks and were sustained for 12 weeks in the patients treated with UBX0101 1.0, 2.0, or 4.0 mg.

In summary, based on the clinically significant and durable improvements in pain and function observed in the Phase 1 study, coupled with the generally well-tolerated nature of UBX0101 IA injection, the benefit:risk profile is favorable. The Phase 1 results support further evaluation of UBX0101 doses of 0.5, 2.0, and 4.0 mg in a Phase 2 study.

2.2 Study Rationale

Building on the favorable clinical responses and safety results obtained during the Phase 1 study, this Phase 2 study is intended to assess the efficacy, safety, and tolerability of a single-dose IA administration of UBX0101 in moderate to severe painful established femoro-tibial OA.

3 OBJECTIVES AND ENDPOINTS

Objectives and endpoints for this study are listed in Table 2.

Table 2 Study Objectives and Endpoints

Tier	Objectives	Endpoints
Primary	Evaluate the effect of IA administration of UBX0101 on the change from baseline to Week 12 of pain in the target knee	Change from baseline to Week 12 of the WOMAC-A score in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo
Secondary	Evaluate the effect of IA administration of UBX0101 on the change from baseline to Week 12 in physical function	Change from baseline to Week 12 of the WOMAC-C function subscale score in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo
Secondary	Evaluate the effect of IA administration of UBX0101 on the change from baseline to Week 12 in pain reported by patients daily over the 12-week study period	Change from baseline to Week 12 of the weekly mean of the average daily pain intensity scores on the 11-point NRS in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo
Secondary	Evaluate the effect of IA administration of UBX0101 on the duration of symptom improvement (pain and function) following IA administration of UBX0101 out to 24 weeks	Change from baseline (over the entire 24-week period, including both the primary study period and the 12-week follow-up period) out to Week 24 for the WOMAC-A, NRS, and WOMAC-C scores in Patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo
Secondary	Evaluate the safety and tolerability of single-dose IA administration of UBX0101	Incidence of AEs as well as change from baseline through Weeks 12 and 24 in vital signs and selected laboratory safety parameters (as deemed clinically appropriate) in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo
Exploratory	Explore the effects of single-dose IA administration of UBX0101 on the change from baseline in patient reported assessment of their osteoarthritis at the time of inquiry	Change from baseline to Week 12 and to Week 24 in PGA score in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo
Exploratory	Explore the effects of single-dose IA administration of UBX0101 on the change from baseline in patient reported impression of how their osteoarthritis has changed over the course of the study	Endpoint: Change from baseline to Week 12 and to Week 24 in PGIC score in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo
Exploratory	Explore the effects of single-dose IA administration of UBX0101 on soluble biomarkers and imaging biomarkers of joint degeneration and repair	Change from baseline out to Week 24 in serum/plasma and urine biomarkers of synovium, cartilage, and bone degeneration Change from baseline to Week 24 in MRI-based markers of joint structure

PGA=Patient Global Assessment; PGIC=Patient Global Impression of Change.

4 STUDY PLAN

4.1 Study Design

This is a randomized, double-blind, placebo-controlled, single-dose, parallel-group study to assess the efficacy, safety, and tolerability of a single-dose IA administration of UBX0101 in patients with moderate to severe painful knee OA. Approximately 180 patients will be randomized (1:1:1:1) to one of four treatment groups (approximately 45 patients per group), all administered by IA route at Week 0. The four treatment groups will be enrolled concurrently.

Group 1: Placebo

Group 2: UBX0101 0.5 mg

Group 3: UBX0101 2.0 mg

Group 4: UBX0101 4.0 mg

Approximately 40 patients per group are expected to complete the Week 12 visit, assuming a 10% dropout rate. Study Drug will be administered as a single 8 mL IA injection at Week 0, Day 1. The primary endpoint analysis will be the change from baseline to Week 12 of the WOMAC-A score in patients receiving a single 0.5, 2.0, or 4.0 mg dose of UBX0101 versus those receiving placebo.

4.2 Design Element Rationale

Data from the Phase 1 study of UBX0101 in OA (Study UBX0101-OAR-101) suggest that administration of a senolytic agent to patients with moderate to severe painful knee OA can produce clinically meaningful improvement in pain and physical function that is sustained for at least 12 weeks after a single dose IA injection of UBX0101. This Phase 2 protocol is designed to confirm in a larger sample size the significant and durable treatment effects observed in Phase 1 and to further explore the doses of UBX0101.

Randomized, Double-Blind, Single-Dose, Placebo-Controlled Parallel-Group Design

To minimize selection bias, the study population will be randomly distributed to four parallel treatment groups. The study population will be treated and followed in a double-blinded manner to ensure that neither the patients nor the Investigator and study team evaluating the patients are biased by knowledge of the treatment received. One measure of maintaining the blind is for all Study Drug injections to be prepared as 8 mL volume solutions so that the blinded administrator of the IA injection is administering Study Drug that is matched for volume and clear, colorless appearance. Another measure to maintain the blind and minimize patient and evaluator bias is to perform all assessments uniformly on all patients, e.g., blood draw for measuring plasma UBX0101 level in placebo and UBX0101-treated patients alike.

Placebo Control

Placebo IA added onto stable background oral medications is the comparator in the study, because there is no universally accepted active comparator treatment for the refractory OA population under study. It is essential to include a placebo comparator group in parallel in the study to accurately describe the treatment effect of UBX0101 adjusted for the amount of placebo response, which is well described in clinical studies of arthritis pain. Lastly, the placebo comparator is useful for evaluating the incidence of safety events in the active UBX0101 groups compared to the incidence of events in the placebo group.

Study Duration

The 24-week treatment and follow-up period allows for an assessment of the longer-term durability of the treatment effect of single doses of UBX0101. Results from the Phase 1 study suggested that single-dose administration of UBX0101 2.0 and 4.0 mg led to improvements in pain and function, which were sustained through the last study visit at Week 12.

WOMAC-A Score as the Primary Endpoint

WOMAC-A is a well-established, validated instrument for measuring pain in knee and hip OA that has been commonly used as a primary or major secondary outcome in many OA clinical studies. WOMAC-A has content validity because it is a composite of five questions about different situations when knee OA pain may manifest itself. Analysis of the change from baseline in WOMAC-A in the Phase 1 study suggested a dose response with increasing doses of UBX0101.

5 POPULATION

5.1 Recruitment

Approximately 180 patients are planned for randomization in this study, anticipating approximately 160 patients, representing 40 patients per treatment group, to complete the Week 12 assessments.

5.2 Definitions

Patients officially enter the Screening period after providing informed consent.

A screen failure is a consented patient who has been deemed ineligible on the basis of one or more eligibility criteria or who has withdrawn consent prior to treatment assignment. Patients will be permitted to re-screen on a case-by-case basis, in consultation with the Sponsor's medical monitor.

An enrolled patient is one who has been deemed eligible and has been randomized and treated to a treatment group.

5.3 Inclusion Criteria

To be included in this study, each individual must satisfy all of the following criteria:

1. Patients who are ambulatory with a diagnosis of OA of the knee, as defined by the American College of Rheumatology (ACR) Criteria (based on clinical and radiologic ACR criteria for at least 6 months; see Appendix 1) and who have baseline pain with a mean of ≥ 4 and ≤ 9 on the 11-point (0–10) average daily pain NRS for at least five of seven days during the Screening period.
2. Patients with a KL grade of 1–4 based on central reading of a weight-bearing radiograph of target knee.
3. Patients aged ≥ 40 and ≤ 85 years.
4. Patients are permitted but not required to use an oral NSAID, serotonin and norepinephrine reuptake inhibitors (SNRIs) such as Cymbalta® (duloxetine), tramadol, or acetaminophen, provided that they have been taking a stable dose and regimen of medication for at least 4 weeks prior to Screening.
5. Patients with type 2 diabetes mellitus can be included as long as they are under adequate control (screening hemoglobin A1c ≤ 8.0) and have no evidence or history of diabetic neuropathy.
6. Patients who have the capacity to give informed consent and who are willing and able to comply with all study-related procedures and assessments. Patients who do not have the legal capacity or medical competency to give written informed consent are ineligible for this study; consent via legally authorized representative is not accepted.

5.4 Exclusion Criteria

An individual is ineligible for this study if he or she meets any of the following criteria:

1. Patients with any condition, including laboratory or imaging findings and findings in the medical history or in the pre-study assessments, that in the opinion of the Investigator or the Medical Monitor constitutes a risk or contraindication for participation in the study or that could interfere with the study objectives, conduct, or evaluation or prevent the patient from fully participating in all aspects of the study.

2. Patients with a body mass index (BMI) ≥40 kg/m^2 or a body habitus that precludes the MRI.
3. Patients with clinically significant co-existing conditions of the cardiovascular, renal, gastrointestinal, respiratory, nervous (e.g., neuropathy), metabolic, hematologic, or immune system.
4. Patients with fibromyalgia based on ACR criteria (Wolfe et al. 2010; Wolfe et al. 2011).
5. Patients with any active, known, or suspected systemic autoimmune disease with musculoskeletal involvement (except for vitiligo, residual autoimmune hypothyroidism requiring only hormone replacement, psoriasis not requiring systemic treatment for 2 years, or conditions not expected to recur in the absence of an external trigger) or any history of a systemic inflammatory arthritis, such as rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis or spondylitis, or reactive arthritis.
6. Patients who have received IA treatment in the target knee with steroids or hyaluronic acid derivatives within the last 16 weeks prior to Screening, or with extended-release corticosteroid (e.g., Zilretta®) within the last 20 weeks prior to Screening.
7. Patients who are using a topical NSAID or topical analgesics on the target knee.
8. Patients who are receiving anticoagulants (e.g., low molecular weight heparin or warfarin), factor Xa inhibitors (e.g., Eliquis®/apixaban), or P2Y12 inhibitors (e.g., Plavix®/clopidogrel or ticagrelor).
9. Patients who are receiving chronic oral corticosteroids.
10. Patients who have used opioid analgesics, marijuana or marijuana-derived products (e.g., cannabidiol), and topical capsaicin on the target knee within 8 weeks prior to Screening and at all times during the study.
11. Patients with a history of traumatic knee injury to the target knee, including, but not limited to, patients with meniscal root tear, within 2 years of study entry.
12. Patients who have undergone diagnostic arthroscopy to the target knee in the previous 6 months.
13. Patients who have undergone arthroscopic surgery (including microfracture and meniscectomy) on the target knee in the last 2 years prior to the Screening visit or are anticipated to have arthroscopic surgery on either knee at any time during the study period.
14. Patients with a history of previous total or partial knee arthroplasty.
15. Patients with an effusion at the Screening visit, which, in the opinion of the Investigator following examination and discussions with the patient, requires drainage for symptom relief.
16. Patients who have had regenerative joint procedures on any joint, including, but not limited to, platelet-rich plasma injections, stem cell transplantation, autologous chondrocyte transplantation, or mosaicplasty.
17. Patients with abnormal alanine aminotransferase or aspartate aminotransferase > 2 × upper limit of normal (ULN) at Screening.
18. Patients with bilirubin ≥ 2 × ULN at Screening.
19. Patients with renal dysfunction as defined by a glomerular filtration rate ≤30 mL/min/1.73 m^2 by laboratory testing (Chronic Kidney Disease Epidemiology Collaboration method) (Levey et al. 2009).
20. Patients with active or untreated hepatitis B or C or any history of human immunodeficiency virus infection at Screening.
21. Patients with any known active infections, including suspicion of IA infection and/or predisposition to infections due to immune system compromise.

22. Patients with a history of malignancy within 5 years and/or current evidence of active malignancy, except basal cell carcinoma, squamous cell carcinoma of the skin, and completely excised cervical intraepithelial neoplasia.
23. Patients with a history of drug or alcohol dependence within the last 3 years.
24. Patients with secondary arthritis that involves the target knee or would confound assessments of knee OA, e.g., joint dysplasia, chronic or recurrent crystal-induced arthritis (gout, calcium pyrophosphate deposition disease, podagra, etc.), aseptic osteonecrosis, acromegaly, Paget's disease, Ehlers-Danlos syndrome, Gaucher's disease, Stickler's syndrome, joint infection, hemophilia, hemochromatosis, or neuropathic arthropathy of any cause.
25. Patients with radiographic (plain X-ray or MRI) evidence of disease that would impact the ability of the study to meet its primary or secondary objectives; examples include the following:
    - Patients with a history of osteonecrosis or who are deemed to have or have risk factors for rapid progression of OA, including sub-chondral insufficiency fracture or spontaneous osteonecrosis of the knee
    - Patients with recent fractures (stress, pathologic, or traumatic) defined by the presence of a visible fracture line
    - Patients with cystic lesion 0.5 × the femoral or tibial subchondral plate
    - Patients with a history of pigmented villonodular synovitis
26. Patients who previously received treatment in a UBX0101 study.
27. Patients who have received another investigational drug or investigational vaccine within the last 3 months prior to Screening.
28. Patients who are participating in or are planning to participate concurrently in another investigational drug or vaccine study.
29. Female patients who are pregnant, lactating, or of childbearing potential who do not agree to use highly effective methods of birth control (e.g., progesterone-only hormonal contraception, double barrier, or intrauterine device) for 3 months following the last dose of Study Drug. Postmenopausal females (> 45 years old and without menses for more than 1 year) and surgically sterilized females are exempt from these requirements.
30. Male patients who do not agree to use a highly effective method of contraception for 3 months following the last dose of Study Drug, if sexually active with a female partner of childbearing potential.

5.5 Lifestyle Restrictions

5.5.1 Fluid and Food Intake

There are no restrictions of fluid or food intake during the study. Patients should be encouraged to maintain their usual diet and fluid intake as much as possible, to avoid any potential confounding impact on the study endpoints.

5.5.2 Patient Activity Restrictions

Patients will not be required to be confined during the course of this study; however, on Day 1, the patient will be required to have an electrocardiogram (ECG) collected prior to Study Drug administration. Additionally, on Day 1 of Study Drug administration, patients will remain in the clinical unit until the 2-hour postdose blood collection for PK assessment.

Patients should refrain from moderate exercise or physical exertion for 48 hours prior to entering the study on Day 1 (day of Study Drug administration). After the IA Study Drug injection, patients will be advised to rest, to avoid ambulation as much as possible for the first 4 hours, and to avoid significant exertion for the first 24 hours after IA injection.

6 STUDY CONDUCT

6.1 Study Procedures

6.1.1 Study Screening and Treatment Periods

The Screening period is 35 days, and the treatment and follow-up period is 24 weeks (from randomization and Study Drug administration to the final follow-up visit).

Patients will be followed-up for up to 24 weeks.

Patient clinic visits are scheduled as follows: Screening, Visit 1/Week 0/Day 1, Visit 2/Week 1/ Day 8, Visit 3/Week 2/Day 15, Visit 4/Week 4/Day 29, Visit 5/Week 8/Day 57, Visit 6/Week 12/ Day 85, Visit 7/Week 18/Day 127, and Visit 8/Week 24/Day 169.

The visit window is ± 10 days for Visits 4 through 8 (Weeks 4 through 24).

In consideration of the COVID-19 outbreak, investigative sites and patients affected may conduct some visits remotely. The asterisks (*) below indicate the critical assessments to be completed remotely and the remaining assessments will not be considered as deviations but should be documented as a “missed assessment with Sponsor approval”. If clinical judgement of the investigator and local recommendations allow, patients may complete Week 12 and Week 24 Visits in clinic.

6.1.1.1 Screening Period/Day -35 to -1

- Obtain informed consent
- Review eligibility criteria
- Record demographics
- Review medical history
- Perform medication review (including concomitant medication)
- Administer WOMAC
- Administer Patient Global Assessment (PGA)
- Perform pregnancy test
- Perform urine drug screen
- Perform physical examination
- Perform knee examination
- Measure vital signs
- Perform laboratory tests (hematology and chemistry)
- Perform knee x-ray
- Perform MRI
- Patients to be trained on completing the NRS Daily Pain question on the e-diary

6.1.1.2 Visit 1/Week 0/Day 1

- Review eligibility criteria
- Review medical history
- Randomization
- Perform medication review (including concomitant medication)
- Administer WOMAC
- Administer PGA
- Administer Multidimensional Psychological Questionnaire (MPsQ)
- Perform physical examination
- Perform knee examination
- Measure vital signs
- Perform ECG
- Perform urine pregnancy test
- Perform laboratory tests (hematology and chemistry)
- Conduct blood biomarker sampling
- Conduct urine biomarker sampling
- Administer study drug
- Conduct AE assessment
- Conduct pharmacokinetics blood sampling

6.1.1.3 Visit 2/Week 1/Day 8 (±5 days)

- Perform medication review (including concomitant medication)
- Administer WOMAC
- Perform knee examination
- Measure vital signs
- Perform laboratory tests (hematology and chemistry)
- Conduct AE assessment
- Review NRS Daily Pain response

6.1.1.4 Visit 3/Week 2/Day 15 (±5 days)

- Perform medication review (including concomitant medication)
- Administer WOMAC
- Measure vital signs
- Conduct AE assessment

- Review NRS Daily Pain response

6.1.1.5 Visit 4/Week 4/Day 29 (±10 days)

- Perform medication review (including concomitant medication)*
- Administer WOMAC*
- Measure vital signs
- Perform laboratory tests (hematology and chemistry)
- Conduct AE assessment*
- Review NRS Daily Pain response*
- Conduct blood biomarker sampling
- Conduct urine biomarker sampling

6.1.1.6 Visit 5/Week 8/Day 57 (±10 days)

- Perform medication review (including concomitant medication)*
- Administer WOMAC*
- Measure vital signs
- Conduct AE assessment*
- Review NRS Daily Pain response*
- Conduct urine biomarker sampling

6.1.1.7 Visit 6/Week 12/ Day 85 (±10 days)

- Perform medication review (including concomitant medication)*
- Administer WOMAC*
- Administer PGA*
- Administer PGIC*
- Perform knee examination
- Measure vital signs
- Perform laboratory tests (hematology and chemistry)
- Conduct blood biomarker sampling
- Conduct urine biomarker sampling
- Conduct AE assessment*
- Review NRS Daily Pain response*

6.1.1.8 Visit 7/Week 18/Day 127 (±10 days)

- Perform medication review (including concomitant medication)*

- Administer WOMAC*
- Measure vital signs
- Conduct AE assessment*
- Review NRS Daily Pain response*

6.1.1.9 Visit 8/Week 24/ Day 169 (±10 days), Final Follow-Up

- Perform medication review (including concomitant medication)*
- Administer WOMAC*
- Administer PGA*
- Administer PGIC*
- Perform physical examination
- Perform knee examination
- Measure vital signs
- Perform laboratory tests (hematology and chemistry)
- Perform serum pregnancy test
- Conduct blood biomarker sampling
- Conduct urine biomarker sampling
- Perform knee x-ray
- Perform MRI (scan may occur 14 days prior to Visit 8 visit to allow for scheduling flexibility)
- Conduct AE assessment*
- Review NRS Daily Pain response and de-activate e-diary*

6.1.1.10 Unscheduled Visits and Early Termination

- Perform medication review (including concomitant medication)*
- Administer WOMAC*
- Administer PGA*
- Administer PGIC (Early Termination only)*
- Perform physical examination
- Perform knee examination
- Measure vital signs
- Perform laboratory tests (hematology and chemistry)
- Perform serum pregnancy test
- Conduct blood biomarker sampling

- Conduct urine biomarker sampling
- Conduct AE assessment*
- Review NRS Daily Pain response and de-activate e-diary (Early Termination only)*

6.1.2 Informed Consent

It is the responsibility of the Investigator to obtain signed written consent for the study from each patient prior to participating in the study to provide for the protection of the patients by following applicable regulations and institutional policies and procedures. The Informed Consent Form (ICF) used during the informed consent process must be reviewed by the Sponsor or designee and approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC).

6.1.3 Medical History and Medication Review, Including Concomitant Medications

A detailed medical history will be obtained by the Investigator or qualified designee. This will include a comprehensive medical history and a complete review of systems, with specific attention to inclusion and exclusion criteria. Past medical history deemed by the Investigator as not clinically relevant to the patient's overall health status or to his or her OA will not be captured. Relevant medical history will be recorded in the electronic case report form (eCRF).

Medications taken until the time of randomization will be recorded as part of the patient's medical history. Medications taken after randomization will be recorded as concomitant medications.

Particular attention will be given to analgesic use history.

6.1.4 Pregnancy Test

Serum pregnancy tests will be performed at a central laboratory at Screening and Final Follow-up (Visit 8/ Week 24) or Early Termination. Urine pregnancy (dipstick) tests will be performed predose on the dosing day (Week 0) and ad hoc at physician's discretion (Table 1).

6.1.5 Physical Examination

Physical examination includes assessments of the skin, head and neck, lungs, heart, abdomen, lymph nodes, extremities, and body weight. Height will be measured at Screening only.

Complete physical examinations will be performed by a licensed physician (or a physician's assistant or nurse practitioner) at Screening and Final Follow-up (Visit 8/ Week 24) or Early Termination. Symptom-directed physical examinations are required to be performed as clinically indicated.

6.1.6 Knee Examination

At the Screening visit and Day 1 knee examinations, there will be a detailed examination of the target knee by the Investigator or qualified designee. The target knee will be examined for erythema, warmth, tenderness, swelling, effusion, crepitus, laxity, and range of motion.

6.1.7 Vital Signs

Vital signs (blood pressure, respiratory rate, pulse rate, and oral temperature) will be obtained in the sitting position. The patient must be in the sitting position for 5 minutes prior to obtaining vital signs.

6.1.8 Electrocardiography

Evaluation will be performed prior to administration of Study Drug for baseline reference. Guidance for performing the ECG evaluation will be included in the ECG manual.

6.1.9 Patient-Reported Outcomes

WOMAC, PGA, and PGIC will be conducted prior to vital signs, physical exams, ECGs, and blood draws. Average daily pain NRS will be collected via an e-diary.

- WOMAC
- PGA
- PGIC
- Average daily pain NRS

6.1.10 Patient Phenotyping Questionnaire

Multidimensional Psychological Questionnaire (MPsQ)

- MPsQ will be collected predose at Visit 1/Week 0/Day 1. To be conducted prior to vital signs, physical exams, ECGs, and blood draws.
- The MPsQ is a questionnaire derived from other validated personality questionnaires that evaluates patient's personality, well-being as well as attitudes and beliefs on disease therapies. It evaluates the likelihood of placebo response.
- MPsQ is self-reported by each patient and each item is rated on a 5-point scale ranging from 1 (strongly disagree) to 5 (strongly agree). The MPsQ used in this study includes 119 questions that are divided into 2 parts to allow some break between parts. Approximately 35 minutes are needed to complete the MPsQ in its entirety.

6.1.11 PK Blood Samples

On Week 0, blood will be drawn for UBX0101 plasma concentrations at 2 hours postdose with a ±15-minute window.

Samples must be collected, processed, and shipped in accordance with the instructions in the Laboratory Manual.

6.1.12 Biomarkers: Blood and Urine

The Laboratory Manual contains detailed instructions for the collection and preparation of samples, directions with respect to the utilization of specialized tubes and requirements for dispensing of aliquots, and storage and shipment of samples to the Sponsor's nominated central laboratory.

6.1.12.1 Blood Collection for Biomarker Analysis

Whole blood to yield plasma and serum samples will be collected per standard of care and will be processed and shipped according to instructions provided in the Laboratory Manual.

6.1.12.2 Urine Collection for Biomarker Analysis

Samples must not be from the first morning void. Urine biomarker sample collection, processing, and shipping instructions can be found in the Laboratory Manual.

6.1.13 Imaging Biomarkers

MRI scans and fixed flexion, standing radiographs of the knee will be obtained at Screening and the Final Follow-up (Visit 8/Week 24) and used to perform exploratory biomarker analyses. Acquisition parameters, transmission instructions, reading, and analyses will be stipulated in the Imaging Manual and/or Imaging Charter.

The imaging sites will be qualified to ensure research conversance and suitability of facilities. The imaging site personnel will be trained by imaging experts prior to initiation of the study. Imaging data will be redacted to ensure that patient identifiers are removed, and the image will be transmitted to a core imaging laboratory, stored in a central imaging database for the study and assessed by central expert reader(s).

6.1.14 End of Study

The study will be considered complete when the last patient has completed the last study visit.

6.2 Discontinuation or Withdrawal

6.2.1 Individual Patients

6.2.1.1 Withdrawal from Study

Patients can voluntarily withdraw from the study for any reason at any time. They are to be considered withdrawn if they state an intention to withdraw, fail to return for visits, or become lost to follow-up for any reason. Patients withdrawing from the study because of an AE should be followed for at least 30 days, until resolution of the AE, or until no further improvement is expected, whichever comes first. Patients withdrawing from the study should be encouraged to complete all assessments under the Unscheduled/Early Termination visit described in Table 1.

6.2.1.2 Replacement of Patients

Patients who drop out of the study prior to the data cutoff for the primary endpoint will not be replaced.

6.3 Study Termination

The Sponsor may suspend or terminate the study or any part of the study at any time for any reason. If the Investigator suspends or terminates that site's participation in the study, the Investigator will promptly inform the Sponsor and the IRB/IEC and provide a detailed written explanation. Upon study completion, the Investigator will provide the Sponsor with final reports and summaries as required by regulations. Upon study suspension, completion, or termination, the Investigator will return all Study Drug, ePRO devices, and other study materials to the Sponsor or designee or destroy the materials at the investigative site per the Sponsor's instructions.

7 STUDY INTERVENTIONS

7.1 Description of Products

The Study Drugs (UBX0101 and placebo) to be provided to the clinical sites will be bulk packaged (sites will be supplied with adequate overage, which will be accounted for as part of Study Drug accountability). The term "Study Drug" when used throughout this protocol means UBX0101 in solution or placebo. Each vial and carton will be individually labeled. Study Drug labeling will include identification required by local law, drug identification, and dosage. The packaging and labeling of the Study Drug will be in accordance with the Sponsor's standards and local regulations. The Study Drugs are manufactured by Vetter Development Service.

7.1.1 UBX0101

7.1.1.1 Formulation, Storage, Preparation, and Handling

The Study Drug will be prepared by an unblinded pharmacist in accordance with the Pharmacy Manual. One or more qualified injectors (e.g., Principal Investigator or Sub-Investigator) will be designated at the site. The injectors will remain blinded. The unblinded pharmacist will not be permitted to interact with patients or conduct any study assessments. Full guidance for Study Drug preparation and administration will be given in the Pharmacy Manual. Documentation of this administration will be retained in the site file and will also be recorded in the eCRFs.

Storage and Accountability

UBX0101 0.5 mg/mL is formulated as a sterile "index solution" at a concentration of 0.5 mg/mL solution in phosphate-buffered saline with 0.4% Polysorbate 80, pH 7.4. Each vial contains 0.5 mg/mL of UBX0101 and is suitable for single-use only. UBX0101 should be stored at -20°C upon receipt. See further details in the UBX0101 Investigator's Brochure (IB).

Storage and dose preparation of study drug will be conducted in accordance with instructions in the Pharmacy Manual and under aseptic conditions.

The Investigator is responsible for drug accountability at the investigational site, appointing a qualified individual to oversee the storage, preparation, and dispensing of Study Drug, and keeping records of such activity in accordance with the requirements of the Sponsor.

All used and unused Study Drug must be stored at site and stored in accordance with the directions given in the Pharmacy Manual.

7.1.1.2 Dosing and Administration

Study Drug will be provided to the responsible pharmacist at the investigational site along with a Pharmacy Manual, which details clinical product presentation and dilutions to be prepared under aseptic conditions.

UBX0101 will be provided in sterile glass vials. Sterile diluent (phosphate-buffered saline with 0.4% Polysorbate 80, pH 7.4) will be provided along with sterile syringes and sterile needles for the preparation of the Study Drug. The Study Drug will be prepared by the unblinded pharmacist based on the patient's treatment allocation as assigned in the randomization system and according to the Pharmacy Manual.

The Study Drug, UBX0101, is administered under ultrasound guidance as a single IA injection in a total volume of 8 mL and will be administered using the following regimens based on treatment group assignment:

Group 2: UBX0101 0.5 mg IA at Week 0

Group 3: UBX0101 2.0 mg IA at Week 0

Group 4: UBX0101 4.0 mg IA at Week 0

7.1.2 Placebo – Phosphate-Buffered Saline

7.1.2.1 Formulation, Storage, Preparation, and Handling

Placebo is the same as the diluent and will be provided as a sterile solution of phosphate-buffered saline with 0.4% Polysorbate 80, pH 7.4, in 10 mL glass vials (see Section 7.1.1.1).

7.1.2.2 Dosing and Administration

The placebo is administered under ultrasound guidance as a single 8 mL IA injection and will be administered using the following regimen based on treatment group assignment:

Group 1: Placebo IA at Week 0

7.2 Treatment Assignment and Bias Minimization

7.2.1 Treatment Allocation

This study will be double-blind and placebo-controlled; the Investigators, study personnel, and patients will be blinded to the treatment assignment. A designated site pharmacist will be unblinded in order to prepare the Study Drug and placebo for administration. The site pharmacist will have access to the treatment allocation through a specialized role within the randomization system, which has role-based permissions. Procedures for maintaining the blind are covered in Section 7.2.3.

7.2.2 Randomization Strategy and Procedure

Patients will be randomly allocated to receive treatment through the randomization system, which administers the randomization code generated by the Sponsor's biostatistician or designee.

A randomization schedule will determine the allocation of either active Study Drug or placebo for each patient by group. Patients randomized to the placebo group (Group 1) will receive a single placebo IA injection at Week 0. Patients in Group 2 will receive a single 0.5 mg IA injection of UBX0101 at Week 0, patients in Group 3 will receive a single 2.0 mg IA injection of UBX0101 at Week 0, and patients in Group 4 will receive a single 4.0 mg IA injection of UBX0101 at Week 0 (Figure 1). Each injection will be a total of 8 mL.

7.2.3 Extent and Maintenance of Blinding

This study will be double-blind; Investigator and Patient will be blind to the treatment assigned. However, the site pharmacist will be required to prepare Study Drug and will therefore have knowledge of the treatment code; the site pharmacist is otherwise not involved in any study

procedures. Sponsor personnel or any designee who is actively engaged with the site will remain blinded.

Treatment will be prepared and dispensed by the unblinded site pharmacist in accordance with the randomization schedule provided by the Sponsor and administered electronically through the randomization system. The site pharmacist may not be involved with the study procedures, assessments, or data recording and will not reveal the randomization code to anyone. The Study Drug will be administered to the target knee through IA injection by an appropriately qualified blinded injector (this individual may be the Investigator or a nominated Sub-Investigator) in the clinic.

It is of importance to ensure that patients are blind to the treatment received; it is recognized that the method of administration of IA injection has the propensity to contribute to a placebo response (Altman et al. 2016), and therefore, maintaining the blind for patients, Investigators, and study personnel who assess patients will assist in minimizing this bias.

7.2.4 Unblinding Procedures

7.2.4.1 Planned Unblinding

Not applicable

7.2.4.2 Unplanned or Unintentional Unblinding

The randomization system will be used to manage roles and permissions with respect to the ability to break the blind. If the Investigator must know immediately what treatment the patient received in order to provide adequate medical care, the code for that patient may be obtained by breaking the blind in the randomization system, which will require recording of the reason for unblinding the treatment allocation.

The Investigator must contact the Medical Monitor to discuss any need to unblind the treatment allocation for a patient in the study prior to unblinding, unless medical emergency dictates otherwise. The randomization code and records regarding any unblinding events will form part of the essential study documents.

A patient who has unplanned or unintentional unblinding during the study will be asked to continue in the study through Week 24 for safety follow-up (AE and concomitant medication reporting, vital signs, physical examinations, and laboratory testing) as well as urine/serum biomarker specimen collections and knee imaging. Patient-reported data will be excluded from efficacy analyses from the point of unblinding onward.

7.3 Assessment and Verification of Compliance

The Study Drug will be managed by the unblinded pharmacist and verified at the investigational site by an unblinded clinical research associate (CRA) or an appropriately qualified designated member of the study team. A Pharmacy Manual will be supplied to the site, and all Study Drug will be required to be accounted for on appropriate drug accountability forms, which will be reviewed by an independent pharmacist. On preparation of the required dose for each patient, the pharmacist or designee will record the detail of vials utilized and retain the overage of the prepared solutions, if any.

7.4 Prior and Concomitant Therapies

7.4.1 Prohibited Therapies

Patients are prohibited from receiving any IA or topical treatment to the target knee during the course of the study, such as:

- IA viscosupplementation or steroid injections to either target or contralateral knee
- Topical NSAID
- Topical capsaicin
- Lidocaine patch

Systemic treatments

- Oral corticosteroids for more than 2 weeks
- Except for tramadol, all other opioid analgesic use (such as oxycodone or codeine containing agents) up to 8 weeks prior to Screening and at all times during the study
- Other investigational drugs by any route of administration
- Anticoagulants (e.g., low molecular weight heparin or warfarin), factor Xa inhibitors (e.g., Eliquis®/apixaban), or P2Y12 inhibitors (e.g., clopidogrel, ticagrelor)

Alternative and non-pharmacological therapies that are prohibited during the course of the study include the following:

- Medical marijuana or marijuana-derived products
- Acupuncture in the target knee

7.4.2 Permitted Therapies

Patients will be permitted to use all regular medications that are not explicitly excluded by the protocol (see Section 7.4.1). Additionally, patients are permitted to use NSAIDs, SNRIs, acetaminophen (paracetamol), and mild opioid analgesic during the course of the study, as long as their dosing is stable at least 4 weeks prior to Screening and during the study period. Any variance from this stable dosing regimen will be recorded in the eCRF Concomitant Medication Log. Variation of NSAID or other analgesic use could impact the interpretation of patient-reported outcomes of pain and function during the course of the study. If it becomes clinically necessary to treat worsening of OA symptoms during the study, it is preferable to adjust medications in the existing regimen over prescribing new analgesic or anti-inflammatory medications.

The use of topical or subcutaneous lidocaine or ethyl chloride topical spray during the IA administration procedure is permitted.

Patients who are taking prophylactic low-dose aspirin prior to Screening may continue on their regular regimen of this therapy during the Screening and the study period.

Patients who are taking metformin concomitantly should be maintained on a stable regimen during the study, as there is some evidence that metformin has beneficial effects on OA (Mohammed et al. 2014; Wang et al. 2019).

Permitted alternative and non-pharmacological therapies include the following:

- Nutritional supplements are permitted if they were a part of the patient's established regimen at least 6 weeks prior to Screening and the patient is committing to maintaining a consistent regimen during the study. Nutritional supplements taken must be recorded in the Concomitant Medication Log.
- Heat and cold therapy: Patients are permitted to use their usual practice of heat and cold therapy, but any variance from usual practice should be recorded in the Concomitant Medication Log.

Assistive devices are permitted during the study, provided the following are observed:

- The use of assistive devices at baseline will be recorded in the electronic data capture (EDC).
- Any change in the use of assistive devices during the course of the study will be recorded in the EDC as a measure of change.

8 SAFETY MONITORING

8.1 Definitions

- **AE**: An AE is any untoward medical occurrence associated with the use of an intervention in humans whether or not it is considered intervention related.
- **Suspected unexpected serious adverse reaction** (SUSAR): A serious AR that is unexpected based on current product information.
- **SAE**: An event is considered "serious" if, in the view of either the Investigator or the Sponsor, it results in any of the following outcomes:
  - Death
  - A life-threatening AE (An event is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the patient at immediate risk of death. It does not include an AE or suspected AR that, had it occurred in a more severe form, might have caused death.)
  - Inpatient hospitalization or prolongation of existing hospitalization
  - A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
  - A congenital anomaly/birth defect
  - Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
- **Causality or relatedness**: AEs should be considered probably or possibly treatment-related, unless they fulfill the following criteria (in which circumstances it should be considered unlikely related or unrelated):
  - Evidence exists that the AE has an etiology other than the Study Drug (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication), and/or
  - The AE has no plausible temporal relationship to administration of the Study Drug (e.g., a new cancer diagnosed 2 days after first dose of Study Drug).

  Relatedness to Study Drug will be graded as "probably", "possibly", "unlikely", or "unrelated" as follows:

  Probably related: **The AE**

  - Follows a reasonable temporal sequence from drug administration
  - Abates upon discontinuation of the drug
  - Cannot be reasonably explained by the known characteristics of the patient's clinical state

  Possibly related: **The AE**

  - Follows a reasonable temporal sequence from drug administration
  - Could have been produced by the patient's clinical state or by other modes of therapy administered to the patient

Unlikely related: **The AE**

- Is most likely to be explained by the patient's clinical state or by other modes of therapy administered to the patient

Unrelated: **The AE**

- Does not follow a reasonable sequence from drug administration
- Is readily explained by and considered by the Investigator to be an expected complication of the patient's clinical state, concurrent medical conditions, or by other modes of therapy administered to the patient

- **AR**: An AR is any AE caused by a drug.
- **Suspected adverse reaction** (SAR): An SAR is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of the investigational new drug (IND) safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. SAR implies a lesser degree of certainty about causality than AR.
- **Unexpected**: An event is considered unexpected if it is not listed in the IB, is not listed at the specificity or severity that has been observed, or, if an IB is not required or available, is not consistent with the risk information described in the General Investigational Plan or elsewhere in the IND. Unexpected also refers to events that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.
- **Severity or intensity:** The severity of an event describes the degree of impact upon the patient and/or the need for medical care necessary to treat the event. AEs reported for patients participating in this study will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (NCI CTCAE v4.03). The Investigator will grade the severity of each AE using, when applicable, the NCI CTCAE v4.03. For AEs not included in the NCI CTCAE v4.03, the criteria outlined in Table 3 should be used as a general guideline.

Table 3 Grading for Adverse Events Not Covered in the NCI CTCAE

Severity	Description
Grade 1 – Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2 – Moderate	Minimal, local or noninvasive intervention indicated; limited age-appropriate instrumental ADL
Grade 3 – Severe	Medically significant but not life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
Grade 4 – Life-threatening	Life-threatening consequences; urgent intervention indicated
Grade 5 – Fatal	Death

ADL=Activities of daily living; NCI CTCAE=National Cancer Institute Common Terminology Criteria for Adverse Events.

8.2 Documenting Adverse Events

8.2.1 Timeframe for Collection

AEs and SAEs will be collected from the time of randomization and treatment. All events prior to this will be documented as medical history.

The Investigator must follow up on all AEs through Week 24 or the last study visit if the patient withdraws early. Nonserious AEs may be followed to resolution past the patient's last study visit at the discretion of the Investigator and/or Medical Monitor if in the best interest of the patient and the assessment of safety of UBX0101.The Investigator must follow up on all SAEs until the events have subsided, returned to baseline, or, in case of permanent impairment, the condition stabilizes.

8.2.2 Classification of Events

Although AEs should be based on the signs or symptoms detected during the physical examination and on the clinical evaluation of the patient, a specific diagnosis should be reported as the AE whenever feasible. In addition to the information obtained from those sources, the patient should be asked the following nonspecific question: "How have you been feeling since your last visit?" Signs and symptoms should be recorded using standard medical terminology.

8.3 Reporting Adverse Events

All AEs and SAEs must be recorded on source documents and collected in the EDC. Any unanticipated risks to the patients must be reported by the Investigator promptly to the Sponsor and IRB/IEC.

All SAEs, whether or not deemed drug-related or expected, must be reported by the Investigator or qualified designee within 24 hours of first becoming aware of the event. The Investigator or qualified designee will enter the required information regarding the SAE into the appropriate module of the eCRF, which will automatically result in distribution of the information to the Sponsors Pharmacovigilance provider. If the eCRF system is temporarily unavailable, the event, including the Investigator-determined causality to study drug, should be reported via fax using an SAE form to the appropriate Sponsor or Pharmacovigilance contact. Upon return of the availability of EDC system, the SAE information must be entered on the SAE eCRF.

The Sponsor (or designee) will process and evaluate all SAEs as soon as the reports are received. For each SAE received, the Sponsor will make a determination as to whether the criteria for expedited reporting have been met. The Medical Monitor should also be contacted immediately for any fatal or life-threatening SAE that is considered possibly or probably related to Study Drug.

The Sponsor (or designee) is responsible for reporting relevant SAEs to the relevant regulatory authorities and participating Investigators, in accordance with Food and Drug Administration regulations 21 Code of Federal Regulations 312.32, International Conference on Harmonisation (ICH) Guidelines, European Clinical Trials Directive (Directive 2001/20/EC, and/or local regulatory requirements and monitoring the safety profile of the Study Drug. To meet this requirement, the Sponsor (or designee) may request additional information from the sites including, but not limited to, hospitalization records, discharge summaries, or autopsy reports. Any requests for such information should be addressed in a timely manner. Additionally, any SAE considered by an Investigator to be possibly or probably related to the Study Drug that is brought to the attention of the Investigator at any time outside of the time period specified for

SAE reporting also must be reported immediately to one of the individuals listed on the Sponsor contact information page.

Reporting of SAEs by the Investigator to the IRB or IEC will be done in accordance with the standard operation procedures and policies of the IRB/IEC. Adequate documentation must be maintained showing that the IRB/IEC was properly notified.

8.4 Adverse Events of Special Interest

No AEs of special interest have been identified for UBX0101.

8.5 Clinical Laboratory Findings

Clinical laboratory tests will include the analytes in Table 4. Patients should be in a seated or supine position during blood collection.

Table 4 Laboratory Parameters

Hematology:	Serum Chemistry:
• Hematocrit • Hemoglobin • Platelet count • RBC count • Mean corpuscular volume • WBC count with differential	• Albumin • Alkaline phosphatase • Alanine aminotransferase • Aspartate aminotransferase • Blood urea nitrogen • Calcium • Carbon dioxide • Chloride • Creatinine[1] • Globulin • Glucose • Hemoglobin A1c (Screening)[2] • Human chorionic gonadotropin[3] • Lactate dehydrogenase • Phosphorus • Potassium • Sodium • Total and direct bilirubin[4] • Total cholesterol • Total protein • Hepatitis B, Hepatitis C, Human Immunodeficiency Virus serology (Screening Only) • Urine Drug Screen (Screening Only)

CKD-EPI= Chronic Kidney Disease Epidemiology Collaboration; RBC=red blood cell; WBC=white blood cell

[1] Creatinine clearance will be calculated by the CKD-EPI method (Levey et al. 2009).

[2] Patients with type 2 diabetes mellitus; performed as a reflex

[3] Serum human chorionic gonadotropin is required only for females who are of childbearing potential.

[4] Direct bilirubin is only required if total bilirubin is above the upper limit of normal.

8.6 Pregnancy

Although not considered an SAE, cases of pregnancy exposure by parent to the Study Drug must be recorded, reported, and followed up as indicated for an SAE. After the patient has been enrolled in the study and received Study Drug by IA injection, the Investigator must report immediately (within 24 hours or next business day whichever is the shorter) any drug exposure during pregnancy to the Sponsor using the Sponsor-supplied Pregnancy Reporting Forms, using the same contact method for SAE reporting. Information about exposure in pregnancy encompasses the entire course of pregnancy and delivery and perinatal and neonatal outcomes, even if there were no abnormal findings. All reports of pregnancy must be followed for information about the course of the pregnancy and delivery, as well as the condition of the newborn. When the newborn is healthy, additional follow-up is not needed. Pregnancies occurring up to 12 weeks after administration of the Study Drug must be reported to the Investigator.

8.7 Overdose or Misuse

Although not considered an SAE, cases of overdose (e.g., a dose higher than that indicated in the protocol, with or without an AE) must be recorded, reported, and followed up as indicated for an SAE.

9 STATISTICAL ANALYSES

9.1 Hypothesis Testing

Building on the favorable clinical responses and safety results obtained during the Phase 1 study, this Phase 2 study is intended to assess the efficacy, safety, and tolerability of a single-dose IA administration of UBX0101 in moderate to severe painful established femoro-tibial OA.

The hypothesis attached to the primary endpoint of this Phase 2 study is that UBX0101, when given as a single IA injection, is related to clinically significant changes in WOMAC-A (pain) compared to a single IA injection of placebo out to 12 weeks post-dose. Nested in the analyses to prove or disprove this hypothesis are factors such as dose response, magnitude, and duration of effect.

9.1.1 Sample Size Determination

Each of the four arms of the Phase 2 design are planned to have approximately 45 patients per group. The target number of patients per group randomized and treated was chosen to facilitate 40 patients per group completers for the 12 week primary endpoint of WOMAC-A score assuming a 10% dropout rate. The sample size was calculated based on Phase 1 WOMAC-A effect size and variability as measured by the mean and standard deviation of the least square mean differences between the treated groups and placebo at Week 12.

In the Phase 1 study, which included five to six UBX0101-treated patients per arm, there was an observed WOMAC-A effect size compared to placebo ranging from 0.2 to 0.7 and a standard deviation ranging from 0.2 to 0.4 on the 0–4 item subscore.

The sample size estimation for Phase 2 was a result of assuming a power of 90% and an alpha of 0.10 for a two-sample, two-sided comparison with an effect size of 0.50 (treated versus placebo) on the WOMAC-A item score (0–4) least square mean at 12 weeks with an assumed standard deviation of 0.75 to account for increased variability in a larger sample size, greater number of sites, and broader patient population under study compared with the previous Phase 1 study.

9.1.2 Analysis Population

9.1.2.1 Demographics

Demographic variables (e.g., sex, race, age, and BMI) will be listed and summarized using descriptive statistics for the entire study population and for each treatment.

9.1.2.2 Baseline Characteristics

Baseline characteristics relevant to the severity of disease will be summarized as described in the Statistical Analysis Plan.

9.1.2.3 Intent-to-Treat Population

All patients who are randomized will be included in the Intent-to-Treat (ITT) population, and their data will be included in efficacy and exploratory data sets. Full reporting of deviations from randomization, noncompliance with study procedures, and protocol deviations will be captured. Data from the ITT population may be used to better understand the prognostic balance of the randomized population and if noncompliance or dropout from the study may be related to their treatment assignment and/or response to treatment.

9.1.2.4 Modified Intent-to-Treat Population

The Modified-Intent-to Treat (mITT) population is a subset of the ITT population who have been randomized and treated. Patients in the mITT population and their data will be included in the safety as well as the efficacy and exploratory data sets.

9.1.2.5 Per-Protocol Population

The Per-Protocol population is a subset of the ITT population who have completed the study without any major protocol violations and who satisfy the requirements for complete outcome data to enable analysis of the outlined primary, secondary, and exploratory endpoint analyses as described in the Statistical Analysis Plan.

9.1.3 Analysis of Efficacy Endpoints

The change from baseline to Weeks 12 and 24 of the WOMAC-A, NRS, and WOMAC-C will be analyzed based on details provided in the Statistical Analysis Plan.

9.1.4 Analysis of Exploratory Endpoints

Exploratory analyses such as evaluation of possible changes in biomarkers related to cartilage and bone degeneration will be outlined in the biomarker analysis portion of the Statistical Analysis Plan.

9.1.5 Safety Analysis

Treatment-emergent AEs and their frequency will be summarized. All AEs will be coded using Medical Dictionary for Regulatory Activities. Summary statistics and plots will be generated for the change from baseline values in the vital signs and selected laboratory safety parameters, as deemed clinically appropriate. Depending on the safety parameter, the difference from baseline will be computed either on the original scale (raw change from baseline) or on the log scale and back-transformed for reporting (percent change from baseline). Summary statistics for the raw laboratory safety tests and/or vital signs may also be computed, as deemed clinically appropriate.

9.2 Planned Interim Analysis

No formal interim analysis is planned.

9.3 Procedures for Reporting Changes to the Planned Analysis

Additional details regarding the planned analyses, and any changes to those analyses described in this protocol, will be provided in the Statistical Analysis Plan.

10 ETHICAL CONSIDERATIONS

10.1 Good Clinical Practice

This study will be conducted in compliance with the protocol approved by the IRB/IEC, and in accordance with ICH Good Clinical Practice (GCP) standards. Any amendments to the protocol or changes to the consent document will be approved by the IRB/IEC before implementation of that amendment. The study will be conducted in accordance with the ethical principles which have their origins in the Declaration of Helsinki.

10.2 Ethics Review

The study and any amendments will be reviewed by an appropriately constituted and composed IRB/IEC. Written IRB approval for the Protocol, amendments, ICF, and Investigator(s) will be obtained in accordance with GCP. The IRB/IEC will be notified of SAEs in accordance with IRB/IEC Policy.

10.3 Informed Consent

An initial sample ICF is provided for the Investigator and IRB/IEC to prepare the informed consent document to be used at his or her site. The site-specific informed consent document will be submitted for review to the central IRB and the IRB-approved informed consent document will be held in the site study file and in the Sponsor's Trial Master File.

The ICF is to be prepared in the language(s) of the potential patient population for this study. The languages under consideration are English and Spanish.

Before a patient's participation in the clinical study, the Investigator is responsible for obtaining written informed consent from the patient after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol-specific screening procedures or any Study Drug is administered.

The Investigator is also responsible for asking the patient if the patient has a primary care physician and if the patient agrees to have his/her primary care physician informed of the patient's participation in the clinical study. If the patient agrees to such notification, the Investigator is to inform the patient's primary care physician of the patient's participation in the clinical study. If the patient does not have a primary care physician and the Investigator will be acting in that capacity, the Investigator is to document such in the patient's medical record. The acquisition of informed consent and the patient's agreement or refusal of his/her notification of the primary care physician, if relevant, is to be documented in the patient's medical records, and the ICF is to be signed and personally dated by the patient and by the person who conducted the informed consent discussion. The original signed ICF is to be retained in accordance with institutional policy, and a copy of the signed consent form is to be provided to the patient.

The patient will be provided with any supplemental information regarding MRI as a procedure in accordance with local and national regulations and institutional policy and may be required to sign a supplemental ICF.

10.4 Data Privacy

All study-related laboratory and clinical data gathered in this protocol will be stored in a password-protected database. All patient information will be handled using anonymous identifiers. Linkage to patients' study data is only possible after accessing a password-protected database. Access to the database is only available to individuals directly involved in the study.

Patient personal health information that is accessed for this study will not be reused or disclosed to any other person or entity, or for other research.

10.5 Financial Disclosure

In connection with the clinical study described in the protocol, the Investigator certifies that the Investigator will read and answer the Clinical Investigator Financial Disclosure Form truthfully and to the best of Investigator's ability. The Investigator also certifies that, if asked, the Investigator will have any other applicable parties (e.g., Sub-Investigators) read and answer the Clinical Investigator Financial Disclosure Form as a condition of their participation in the study. If the financial interests reported on the Clinical Investigator Financial Disclosure Form change during the course of the study or within 1 year after the last patient has completed the study as specified in the protocol, the Investigator and the other applicable parties are obligated to update the Sponsor of financial disclosure in accordance with the Sponsor's standard procedures.

10.6 Biological Specimens and Data

Biological samples should not be destroyed at the end of the study. Samples should be sent to the Sponsor's designated laboratory as detailed in the Laboratory Manual. Samples may be tested for exploratory endpoints or additional further research after consent has been obtained.

11 OVERSIGHT

11.1 Safety Monitoring

A safety assessment committee will be established to support pharmacovigilance activities for the study, such as adjudication of AEs or possible safety signals. The Safety Assessment Committee Charter will describe the committee's structure, roles, and responsibilities.

11.2 Quality Control and Assurance

11.2.1 Monitoring and Audits

The Investigator will permit regular study-related monitoring by the Sponsor or designee, audits, IRB/IEC review, and regulatory inspections by providing direct access to source data and documents.

11.2.2 Protocol Deviations

Protocol violations/deviations will be documented in accordance with good documentation practice and reported to the IRB/IEC in accordance with IRB/IEC Policy. In case of a deviation necessary to eliminate an immediate hazard to a research participant, the deviation will be reported to the IRB/IEC as soon as possible. Investigational sites should make every effort to adhere to the processes and procedures described in this protocol.

11.2.3 Records

11.2.3.1 Data Capture and Management

An EDC system will be designed and managed on behalf of the Sponsor by the Sponsor's designated contract research organization. Clinical data will be entered by study site personnel within 5 business days of the patient visit or activity conduct. Monitoring of the study will be conducted on site by a designee of the Sponsor (CRAs) who will conduct document and source data review, as well as remote data monitoring in the intervals between monitoring visits. Data will be reviewed remotely by the Medical Monitor for safety oversight.

Investigator

All study-related information will be recorded on source documents. All required study data will be recorded in the eCRFs. All eCRF data must be submitted to the Sponsor throughout and at the end of study in a timely and accurate manner.

If an Investigator retires, relocates, or otherwise withdraws from conducting the study, the Investigator must notify the Sponsor to agree upon an acceptable storage solution.

Regulatory agencies will be notified with the appropriate documentation.

Sponsor

The data will be checked for completeness and correctness in real-time online.

Data are checked as they are entered into the EDC system. Offline checks will also be run to assess the need for additional data review. Discrepancy reports will be generated and transferred to the study center for resolution by the Investigator or its designee.

11.2.3.2 Records Retention

The Investigator shall retain and preserve one copy of all data generated in the course of the study, specifically including, but not limited to, those defined by GCP as essential, for the duration of (i) 2 years after the last marketing authorization for the Study Drug has been approved or the Sponsor has discontinued its research with respect to such Study Drug or (ii) such longer period as required by applicable global regulatory requirements. At the end of such period, the Investigator shall notify the Sponsor, in writing, of the intent to destroy all such material. The Sponsor shall have 30 days to respond to the Investigator's notice, and the Sponsor shall have a further opportunity to retain such materials at the Sponsor's expense.

12 PUBLICATION POLICY

Any formal presentation or publication of data collected from this study will be considered as a joint publication by the Investigator(s) and the appropriate personnel at UNITY Biotechnology.

Authorship of any publications resulting from this study will be mutually agreed and determined on the basis of the Uniform Requirement for Manuscripts submitted to Biomedical Journals (International Committee of Medical Journal Editors), which states the following:

- Authorship credit should be based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or critically revising it for important intellectual content; (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.
- When a large, multicenter group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship defined above.
- Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
- All persons designated as authors should qualify for authorship, and all those who qualify should be listed.
- Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. (Additional information on the current guidelines for publications can be found at the following location: http://www.icmje.org/).
- All publications (e.g., manuscripts, abstracts, oral/slide presentations, or book chapters) based on this study must be submitted to UNITY Biotechnology, for review. The Clinical Trial Agreement among the institution, Investigator, and UNITY Biotechnology will detail the procedures for, and timing of, UNITY Biotechnology's review of publications.

13 FINANCING AND INSURANCE

Financial disclosure, site budget, and any insurance policies relevant to this clinical study are described in each Clinical Trial Agreement.

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15 APPENDICES

APPENDIX 1 DIAGNOSTIC CRITERIA

American College of Rheumatology Criteria for Classification of Idiopathic Osteoarthritis of the Knee

Clinical and Radiographic Criteria (Altman et al. 1986):

- Knee pain and at least 1 of 3:
  - Age > 50 years
  - Stiffness < 30 minutes
  - Crepitus

and

- Osteophytes

APPENDIX 2 PATIENT-REPORTED OUTCOMES: 11-POINT PAIN NRS

During the course of the study, patients will be asked to respond to a daily pain question (adapted from the Brief Pain Inventory) and rate their average pain over the last 24 hours on an 11-point scale:

"Please rate your study knee pain by selecting the number that best describes your study knee pain on average over the past 24 hours"

0 = No pain
10 = Pain as bad as you can imagine

Pain as a PRO will be collected on a daily basis throughout the duration of the study on a handheld electronic device for reporting of daily pain between the hours of 4:00 pm and 11:30 pm.

APPENDIX 3 PATIENT GLOBAL ASSESSMENT AND IMPRESSION OF CHANGE

Patient Global Assessment

Patients will be asked the following question at Screening, Day 1, Week 12, and Week 24 and provided the following numeric rating scale. This question will be administered as a patient facing paper questionnaire.

Considering all of the ways that your knee arthritis affects you, how are you doing today?

Patient Global Impression of Change

Patients will be asked the following question at Week 12 and Week 24 and provided the following multiple-choice responses to select from. This question will be asked verbally by the study coordinator/ appropriate team member at the site during the clinic visit. The patient's response should be recorded in the source and eCRF.

Since having the study treatment on Day 1 at this clinic, how would you describe the change (if any) with treatment to your osteoarthritis of the study knee? Please take into account symptoms, activity/function, and your quality of life in relation to the OA of your study knee.

- ☐ *Very much improved*
- ☐ *Much improved*
- ☐ *Minimal improvement*
- ☐ *No change*
- ☐ *Minimally worse*
- ☐ *Much worse*
- ☐ *Very much worse*