then evaluated the effects of NOX4 deletion on cartilage anabolism and catabolism by IHC. As expected DMM surgery increased catabolism (MMP13 and COL1 expression) and inhibited anabolism (COL2 expression). In comparison NOX4 deletion in mice inhibited MMP13 and COL1 expression and increased COL2 expression after DMM surgery. Then we assessed if the effects observed in the NOX4$^{-/-}$ mice was linked to synovial inflammation and ROS production in the cartilage in vivo (Figure 8). First, we evaluated the expression of NOX4 by IHC. With HE staining, we demonstrated that DMM surgery induced synovial inflammation (increased cellularity and thickness) in the WT mice but not in the NOX4-/- mice. Using synovitis scoring system, we observed an inflammation score of 2.5 ± 0.5 in WT mice versus 0.9 ± 0.4 (p<0,01) in NOX4-/- mice. In accordance with these results, we observed a strong decrease of the expression of the macrophage marker F4/80 in the synovial membranes of the NOX4-/- mice compared to the WT mice. Finally, we demonstrated that NOX4 deletion inhibited 8-OHdG expression in the cartilage and decreased its expression in the synovial membrane.

Conclusions: Our results demonstrated that NOX4 deficiency decreases cartilage degradation and regulate the anabolism/catabolism balance *ex-vivo* after IL-1β stimulation and decreases significantly experimental OA severity in mice. We also showed that NOX4 increase OA severity via chondrocytes ROS production and synovial inflammation. Taken together these results underline that NOX4 could be a major target to dampen OA progression.

25
CHARACTERIZING THE COMBINED EFFECT OF PHYSICAL ACTIVITY MEASURES ON STRUCTURAL PROGRESSION OF OA: THE MULTICENTER OSTEOARTHRITIS STUDY

K.E. Costello [1], A. Guermazi [2], F.W. Roemer [3], C.E. Lewis [4], J.C. Torner [5], M.C. Nevitt [6], D.T. Felson [2], **S. Jafarzadeh** [2]. [1] *Boston Univ. Coll. of Hlth.& Rehabilitation Sci.: Sargent Coll., Boston, MA;* [2] *Boston Univ. Sch. of Med., Boston, MA;* [3] *Univ.sklinikum Erlangen & Friedrich-Alexander Univ. Erlangen-Nürnberg, Erlangen, Germany;* [4] *Univ. of Alabama at Birmingham, Birmingham, AL;* [5] *Univ. of Iowa, Iowa City, IA;* [6] *Univ. of California, San Francisco, San Francisco, CA*

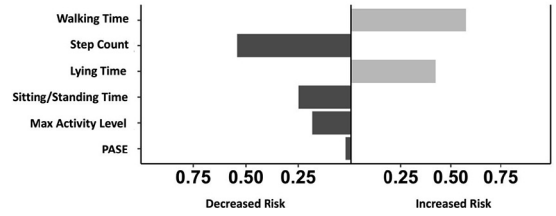
Purpose: Physical activity is consistently recommended as a management strategy for knee osteoarthritis (OA); however, studying the effect of daily physical activity on structural progression of OA remains challenging. Accelerometry data, for example, quantifies activity but does not specify the type of activity or its complexity. Surveys often capture frequency and intensity of various complex activities and provide complementary information to that of accelerometry. Previous studies have used either surveys or accelerometry to assess the relation of physical activity to OA outcomes but not both. Further, analyses of both surveys and accelerometry have usually summarized overall activity when it is likely that different types of activity affect OA differently. It is challenging to infer the overall effect of various activity measures because focusing on one in a model assumes the others remain unchanged (i.e., holding other measures constant), which is unlikely. It is unclear how various measures of physical activity could act synergistically or antagonistically on OA outcomes, which could be important for informing the physical activity prescription. The goal of this study was to use both survey and accelerometry data to quantify the combined effect of physical activity measures on cartilage loss using a causal-inference based approach, and to characterize the direction and effect size for the contribution of each individual physical activity measure.

Methods: We used data from the 144- and 168-month study visits of the Multicenter Osteoarthritis (MOST) Study, a NIH-funded longitudinal cohort of persons with and without knee OA. Magnetic resonance imaging (MRI) was read in one knee per person; thus, data from only one knee from each participant was analyzed. The outcome was defined as a binary (dichotomized) variable representing any 1/2 grade or greater worsening of area and/or depth of medial tibiofemoral cartilage (i.e., increase in semi-quantitative MRI Osteoarthritis Knee Score [MOAKS] score) between 144- and 168-month visits. Physical activity measures included the Physical Activity Scale for the Elderly (PASE) total score and AX3 accelerometer-based physical activity measures (averaged over >= 3 valid wear days): time spent walking, sitting/standing, and lying, step count, and maximum overall activity level based on signal vector magnitude. Confounders measured at 144-month visit included sex, race, body mass index (BMI), Center for Epidemiologic Studies Depression Scale (CES-D), Kellgren and Lawrence (KL) score, hip-knee-ankle alignment, knee injury history, and knee pain quantified by the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) score. We developed a marginal structural model, estimated by quantile g-computation, to quantify the combined effect, referred to as mixture effect, of various physical activity measures on the odds of cartilage loss. For quantile g-computation, we quantized each physical activity measure into half a decile increments (producing 20 levels or "ventiles", each containing 5% of data).

Results: The study sample included 1416 MOST participants (60% female; 13.2% Black or African-American; 144-month visit mean age = 61.0 [SD = 9.1], mean BMI = 28.3 [SD = 4.9]). Of 1416 knees, 249 (17.6%) experienced cartilage loss worsening. Estimate of the mixture effect of physical activity measures suggested that an increase in physical activity measures reduced the odds of cartilage loss over 2 years. Specifically, the odds of cartilage loss decreased by 6% (marginal causal odds ratio = 0.94, 95% CI: 0.87, 1.03) per ventile (i.e., half a decile) increase in physical activity measures. Among physical activity measures, an increase in walking time and lying time components were associated with increased odds of cartilage loss, while an increase in step count, sitting/standing, maximum overall activity level, and PASE scores were associated with reduced odds of cartilage loss (Figure 1).

Conclusions: These results suggest that physical activity, defined using a combination of physical activity measures, reduces the odds of cartilage loss, supporting current guidelines that recommend physical activity as a management strategy for knee OA. The positive impact of step count in the context of a negative impact of walking warrants further exploration of activity pacing and intensity of physical activities.

Figure 1. Scaled effect sizes for the effects of various physical activity measures on cartilage loss



26
IMPROVED WOMAC PHYSICAL FUNCTION IS ASSOCIATED WITH SLOWED PATHOLOGICAL BONE SHAPE CHANGE AFTER TPX-100: TOWARDS A SURROGATE MARKER FOR VIRTUAL KNEE REPLACEMENT?

D. McGuire [1], M. Bowes [2], A. Brett [2], N. Segal [3], M. Miller [1], D. Rosen [1], Y. Kumagai [1]. [1] *OrthoTrophix, Foster City, CA;* [2] *Imorphics, Manchester, United Kingdom;* [3] *Univ. of Kansas Med. Ctr., Kansas City, KS*

Purpose: TPX-100 is a 23-amino acid peptide derived from a small integrin-binding ligand N-linked glycoprotein (*SIBLING*) family member: Matrix Extracellular Phosphoglycoprotein (MEPE). MEPE is highly expressed by osteocytes and may play a role in bone remodeling. Previously reported non-clinical and clinical data support the safety and efficacy of IA TPX-100 in knee OA. In Study TPX-100-5, we were interested in examining clinical and structural measures that have been shown to predict joint failure and total knee replacement (TKR), informed by two recent research streams. The MOST study (N ≈ 2700) demonstrated a strong predictive association between WOMAC Physical Function (Table 1) and risk of TKR. There was a marked pain-independent dose-response relationship for all levels of functional impairment. Poorer scores were associated with increased risk of TKR up to 15 times overall and 5.9 times after controlling for severity of pain. Structurally, MRI-based femoral bone shape (B-score) analysis also has

been shown to predict TKR (Figure 1). The present study was designed to investigate bone-shape changes in subjects with MRI-confirmed, bilateral OA who had received IA TPX-100 or placebo; and to examine relationships, if any, between B-scores and WOMAC Physical Function scores in this population.

Methods: All subjects participated in TPX-100-1 (n=93 subjects,186 knees), a Phase 2 randomized, double-blind, controlled clinical trial of TPX-100 versus saline control. Per the *a priori* statistical analysis plan, all subjects who received 4 weekly injections of TPX-100 (200 mg/injection) in one knee and saline placebo in the contralateral knee were eligible for inclusion in TPX-100-5. MRIs were centrally assessed for quality, blind to treatment assignment and clinical data. Image quality was sufficient in 78 of the 93 subjects (84%) to determine a reliable B-score. Femur bone surfaces were segmented automatically at a single center (Imorphics Ltd, Manchester, UK). An "OA vector" for femur bone-shape analysis was defined, as previously reported, based on over 4,500 subjects' knees from the Osteoarthritis Initiative (OAI). The OA vector is the line that passes through the mean shape of two populations: an OA Group, defined as all knees with KLG 2 or greater over 4 years of follow-up, and a Non-OA Group, defined as knees with KLG of 0 in the same period. Distance along the vector determines a "B-score", with the origin (B-score = 0) marking the mean shape of the Non-OA Group for each sex. One B-score unit represents one standard deviation of the Non-OA Group along the OA vector, moving towards the OA Group. Knees with baseline femur B-scores of 1.5 or greater were selected as representing definite radiographic OA (KLG of 2 or greater), per published criteria. B-scores and WOMAC Physical Function scores in knees receiving TPX-100 versus those receiving placebo were compared from baseline through 12 months.

Results: TPX-100-treated and control knees were similar in all baseline characteristics, including femur B-scores and WOMAC function scores. The demographics of the cohort with B-score of 1.5 or greater were similar to those of the whole study population (Table 2). Physical Function scores improved significantly and meaningfully in favor of TPX-100 at 3 and 12 months (Figure 2). Pathological B-score change was markedly decreased in TPX-100-treated knees at 6 and 12 months compared with placebo-treated knees. Notably, the trajectory of bone-shape change of TPX-100-treated knees was nearly identical to that of "non-OA" knees from the Osteoarthritis Initiative (OAI) data base. Control knees, in contrast, had an increased slope of shape-change trajectory similar to that of "OA knees" (Figure 3).

Conclusions: WOMAC Physical Function scores and B-score assessments provide clinical and structural outcome measures that each predict TKR. When appropriately combined, these measures may provide a surrogate measure, or "virtual TKR", suitable for OA treatment trials. Treatment-related improvements in knee function and reduction in pathological bone shape change may reasonably be expected to reduce the risk of TKR, with its burden of costs and potential complications. In subjects with moderate to severe knee OA, IA TPX-100 is associated with sustained, improvements in WOMAC Physical Function and slowing of pathologic femur bone shape change. These data providing preliminary evidence for the disease-modifying effects of TPX-100, including its potential for delaying or preventing TKR.

Table 1. Questions for WOMAC Physical Function Score.
The questions are asked as follows:
"The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your RIGHT/LEFT knee.

Q1 ~ 15: None (0), Mild (1), Moderate (2), Severe (3), Extreme (4)
Q16 ~ 17: Never (0), Rarely (1), Sometimes (2), Often (3), Always (4)"

Q	Functions
1	Descending stairs
2	Ascending stairs
3	Rising from sitting
4	Standing
5	Bending to floor/pick up an object
6	Walking on flat surface
7	Getting in/out of car
8	Going shopping
9	Putting on socks/stockings
10	Rising from Bed
11	Taking off socks/stockings
12	Lying in bed
13	Getting in/out of bath
14	Sitting
15	Getting on/off toilet
16	Heavy domestic duties
17	Light domestic duties

Table 2. Demographic of Baseline B-score ≥1.5 Knees vs. All TPX-100-5 Knees

Severity Cohort	Index			Control			Mean Age		Mean BMI	
	Male	Female	Female %	Male	Female	Female %	Index	Control	Index	Control
All (N=78)	30	48	62%	30	48	62%	58.4	58.4	30.9	30.9
B score ≥1.5	12	23	66%	13	22	63%	59.9	61.2	32.0	32.6

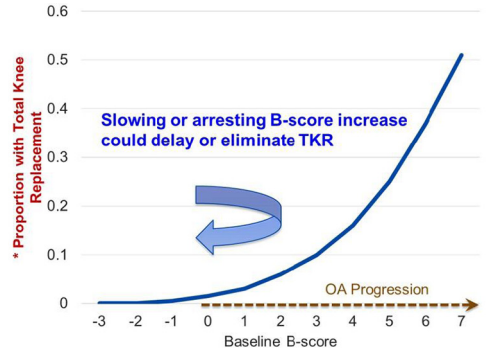


Figure 1. Risk of Total Knee Replacement by B-Score Increase – Data from OAI.
Knees were followed up for up to 8 years, in average 5 years (Bowes et al 2020).

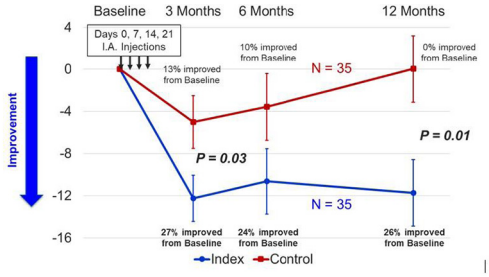


Figure 2. TPX-100 showed statistically significant reduction in WOMAC Function as compared to control in the knees with baseline B score ≥ 1.5.
Mean baseline scores were 44.8 and 37.1 of 100, respectively (p = 0.14). Error bars indicate standard errors of the mean. Statistical analyses were by Student's t-test (unpaired and unequal variance).

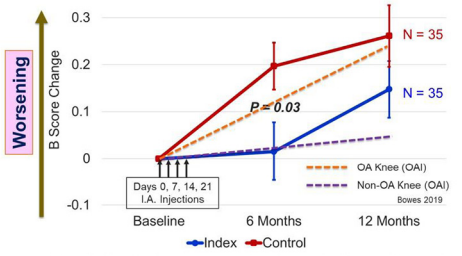


Figure 3. TPX-100 significantly reduced pathological femoral bone shape (B score) change as compared to control in the knees with baseline B score ≥ 1.5.
Mean baseline B scores of Index and Control knees were 3.08 and 3.02, respectively. Error bars indicate standard errors of the mean. Statistical analyses were by Student's t-test (unpaired and unequal variance).

V-27
ASSOCIATEION BETWEEN LEVOTHYROXINE USE AND LONGITUDINAL THIGH MUSCLES QUALITY AND RISK OF KNEE OSTEOARTHRITIS INCIDENCE: OSTEOARTHRITIS INITIATIVE DATA

B. Mohajer [1], K. Moradi [2], M. Dolatshahi [2], A. Guermazi [3], F. Roemer [3,4], S. Demehri [1]. [1] *Johns Hopkins Univ., Baltimore, MD;* [2] *Tehran Univ. of Med. Sci., Tehran, Iran, Islamic Republic of;* [3] *Boston Univ. Sch. of Med., Boston, MA;* [4] *Univ. of Erlangen-Nuremberg, Erlangen, Germany*

Purpose: Hypothyroidism can commonly cause myopathy, characterized by myalgia and weakness, affecting almost 80 percent of patients. This myopathy may be important in the course of knee osteoarthritis (KOA), in which thigh muscles play an essential role in the incidence and progression of the diseases. Despite that hypothyroidism and KOA are very common and frequently coexist in the elderly population, no conclusive evidence is available regarding the possible association of hypothyroidism with thigh muscle changes. Therefore, we aimed to assess the association between levothyroxine use, as an