# CYCLIN-past-OA: CDK8/19 inhibition as an osteoarthritis therapeutic strategy

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**Introduction:** Osteoarthritis (OA) is a whole joint degenerative disease characterised by cartilage matrix mineralisation/degeneration, subchondral bone remodelling, osteophyte formation and synovial inflammation. Chondrocyte hypertrophy is a key hallmark of OA that drives cartilage catabolism, in addition to promoting other aspects of joint pathology. There are no approved disease modifying therapeutics, while surgery is often unsuccessful and pain management is suboptimal, thus there is urgent need for novel therapeutics.

**Methods:** We have developed and used a novel *in vitro* screening platform to identify modulators of chondrocyte (ATDC5) mineralisation/hypertrophy via alcian blue and alizarin red staining. Screening a library of epigenetic modulators allowed pinpointing of the CDK8/19 inhibitor (CDK8/19i), which had not been previously associated with OA. Measurement of marker gene expression levels was performed via qPCR and RNAseq, and Mitostress Test (Agilent) was carried out on hypertrophic cells to define effect on metabolism. Primary equine (N=6), canine (N=4) and human (N=1and MSC-derived) chondrocytes were differentiated in the absence/ presence of CDK8/19i for 12 days for chondrogenic gene expression analysis, followed by 48-hour IL1β treatment for OA-like gene expression analysis. To determine effects of CDK8/19 inhibition on other joint resident cells known to be involved in OA progression, THP-1-derived macrophages were LPS-stimulated in the absence/ presence of a CDK8/19i. Gene expression analysis and IL1β cytokine production was measured via qPCR (N=3) and ELISA (N=2) respectively. Additionally, osteoblast-like cells (MC3T3 line) and primary bone marrow progenitors (isolated from C57BL/6 mice and seeded onto dentine discs) were used to determine the effect of CDK8/19i on osteoblast and osteoclast biology, respectively. *In vivo* analysis was initially conducted on 24hpf zebrafish embryos through CDK8/19i supplementation to the water until 5dpf before staining with alcian blue. STR/ort mice (spontaneous OA model) were orally dosed from 22-weeks old for up to 12 weeks (DMSO N=5, CDK8/19i N=6) followed by gait analysis via DigiGate, subchondral bone structural analysis via μCT and serum analysis via ELISA. All procedures were in accord with RVC animal welfare and ethics review board (AWERB) approval in fulfilment of the UK Animals (Scientific Procedures) Act 1986. Statistical significance determined via T-test with Welch correction.

**Results:** Transcriptomic analysis of ATDC5 cells via qPCR and RNAseq revealed that treatment with CDK8/19i increased levels of anti-hypertrophic and pro-anabolic markers ($p < 0.0001$, N=3), including ACAN and COL2A1 (>5-fold), CNMD and TIMP4 (>100-fold) and downregulated hypertrophy markers such as MEF2C (3-fold), IHH (5-fold) and IBSP (68-fold). Biological process enrichment of 921 differentially expressed genes identified cartilage anabolic mechanisms to be enhanced by CDK8/19i treatment. Notably, CDK8/19i had a pro-anabolic effect on human, equine, and canine chondrocytes, and protected from IL-1β induced ECM degradation. Interestingly, CDK8/19i also supressed levels glycolytic activity in hypertrophic cells by 37% ($p < 0.05$), whilst simultaneously reducing inflammatory IL-1β/TNF-α gene expression (10- and 2-fold, respectively, $p < 0.01$) and secretion (IL-1β 1.5-fold $p < 0.05$) in THP-1-derived macrophages. Furthermore, CDK8/19i abolished matrix mineralisation in MC3T3 cells, in addition to impeding osteoclast resorption 2.3-fold ($p < 0.0001$) without modifying osteoclast formation/maturation. *In vivo* analysis of treated 5dpf zebrafish embryos displayed a dose-dependent reduction in cells at a future ossification site, with 100nM CDK8/19i reducing cell count by 1.6-fold ($p < 0.01$, N=8). Finally, *in vivo* treatment of the spontaneous OA STR/ort mice resulted in an increase in anabolic gene expression, including MATN3 (100%), CNMD (69%) and ACAN (43%) in knee cartilage, a modification in subchondral bone parameters and, vitally, an improvement in gait symmetry and treadmill task completion rates ($p < 0.05$). Serum analysis revealed a 2.2-fold decrease in the OA-associated oxidative stress marker, malondialdehyde (MDA; $p = 0.056$) and a trend for reduced IL-1β levels (~2-fold).

**Discussion:** Our data highlight that CDK8/19i modifies whole joint cell behaviour through modification of dysregulated cell phenotype, as observed in OA. CDK8/19i results in a pro-anabolic and anti-catabolic/hypertrophic phenotype in both chondrogenic cells lines, primary cells (multi-specie) and *in vivo* implicating CDK8/19 in the control of chondrocyte phenotype. Additionally, CDK8/19i-mediated inhibition of osteoblast mineralisation and osteoclast resorption is therapeutically relevant for OA, as early subchondral bone remodelling and late stage osteophyte formation are key events that drive represent progression. CDK8/19i also dampens pathological pro-inflammatory mechanisms in multiple joint resident cell types and in *in vivo* serum analysis, suggesting treatment may impede progressive joint damage by inflammatory mediators. Intriguingly, CDK8/19i corrects gait asymmetry and improves treadmill performance in spontaneously osteoarthritic mice, indicative of disease-modifying capabilities.

**Significance/clinical relevance**: Collectively, these data disclose CDK8/19 inhibition as a promising strategy for disease-modification in OA. This is the first report identifying a role of CDK8/19 in chondrocyte biology and OA. Our approach is both unique and timely as it pinpoints inhibition of CDK8/19 as a therapeutic paradigm to control multiple cellular events that are crucial to joint pathology. This CDK8/19i-mediated control of multiple cellular behaviours is particularly appealing as prior targeting of single disease-linked factors (e.g., IL1β and ADAMTS5) has failed.
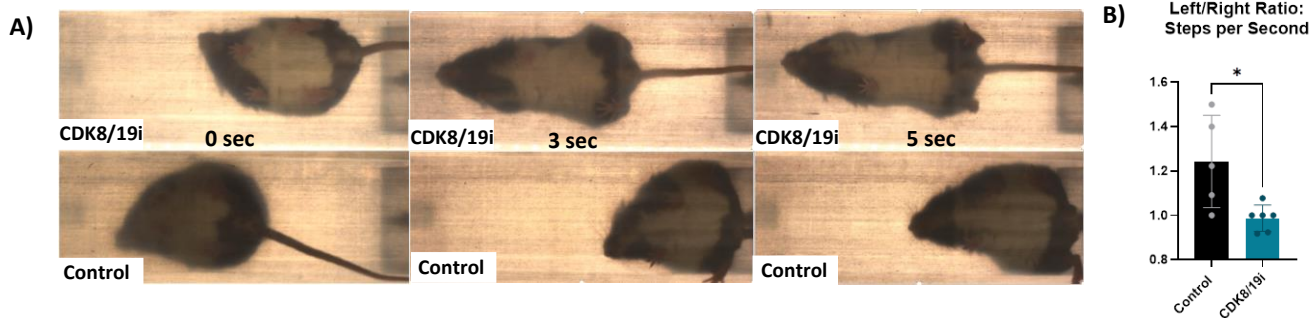
Figure. 1 CDK8/19i improves mobility in the STR/ort mouse model. A) Timelapse snapshot images of a STR/ort mouse treated with CDK8/19i (top) shows treatment improves mouse treadmill performance compared to controls (bottom). B) CDK8/19i treatment (teal, N=6) corrects gait symmetry, normalising the step per leg ratio which is not seen in control (black, N=5, <0.05).