scorewas 977.66 in group A and B 1314.50in group B at week twenty six (26th), which wassignificant p<0.05.
Conclusions: IAHA injection is more beneficial to Naproxen sodium along with exercise and ADL in OA knee.

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DISCOVERY OF POTENTIAL THERAPEUTICS FOR POST-TRAUMATIC OSTEOARTHRITIS USING A HIGH THROUGHPUT MECHANICAL INJURY PLATFORM

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Purpose: The aim of this study was to utilize a high throughput cartilage injury platform that can be used as a model of post-traumatic osteoarthritis (PTOA) and rapidly assess a large number of compounds that are relevant to many cell functions after compressive injury. We developed and validated an *in vitro* high throughput mechanical injury (HiTMI) platform for investigating PTOA using engineered cartilage. The injury response in this model mimics that of native cartilage, and enables screening of chemical libraries for therapeutic discovery in a "high" throughput manner. Here we describe the screening of a number of small molecule compound libraries targeting various pathways using this HiTMI platform. Our goal was to identify 'hits' (i.e. significant regulators) that might serve as novel therapeutics for early intervention in PTOA. Six libraries were evaluated, including natural and synthetic compounds with potential to regulate a variety of cell responses, such as apoptosis, cell signaling, and cell proliferation. We probed compounds libraries that contained protease, kinase, and phosphatase inhibitors, all critical to cell function. An additional natural compound library and one focused on anti-apoptosic molecules was also utilized. Rapidly identifying compounds that may affect cell behavior and health in the acute phase post injury could be timely and of significant clinical importance to this growing form of osteoarthritis.
Methods: Our model consists of engineered cartilage tissue analogs (CTA), which have been extensively characterized in our laboratory. In this model, chondrocytes are cultured in high density cultures above a hydrogel coating (poly 2-hydroxyethyl methacrylate) to prevent cell attachment. Within 24 hours, chondrocytes coalesce to form a stable construct that remains in suspension and increases in mass with time. Chondrocytes in CTAs possess phenotypic characteristics and deposit ECM that is similar to native cartilage. Since CTA can be generated in large numbers (100's), each bearing an identical profile, they were used in our screening studies using a multi-platen mechanical injury device we recently fabricated. This device uses an Instron to deliver a single compressive injury, similar to that which occurs in a clinical setting. We have previously shown this to mimic the injury and cell responses in cartilage explants. CTA (4-5 months) were compressed to 75% strain at a rate of 50% strain/sec. Immediately following injury, CTA were treated with library compounds at 10 υM. Since this is a HTS platform, all primary screenings were carried out for 48 hours post injury/treatment. Medium was collected to assess proteoglycan loss (by measuring released or soluble glycosaminoglycans (GAG) using the DMMB assay) and indirectly measure cell stress / death (by measuring LDH using the CytoToxONE Homogenous Membrane Integrity Assay), two hallmarks of acute cartilage injury. Additional secondary screening was performed on positive hits using early (48 h) and late (96 h) time points and extended dose ranges.
Results: Using this cartilage analog injury platform, we screened a total of 428 molecules. Primary screening resulted in 5 positive hits in the protease inhibitor, 9 in the apoptosis, 8 in the phosphatase, 10 in the kinase, and lastly 12 in the natural compound library (Figure 1). For example, results from the kinase inhibitor library revealed that there were 10 compounds that met the 'hit' criteria based on the released sGAG. These molecules included included PDGFR tyrosine kinase inhibitors. Conversely, for example, one compound, AG-370 resulted in worsening of the response (increased sGAG and LDH). The third phosphatase library screening resulted in identification of potential hits including compounds inhibitors of calcineurin. From 23 compounds screened through secondary screening, and based on a 20% or better threshold, more than 3 of these compounds remain significantly positive with respect to GAG release, relative to the injury-control. The compounds, with activities specific to pan-caspase inhibition, tyrosine phosphatase inhibition, and JNK-pathway signaling activation, resulted in a released-GAG reduction of 43%, 24% and 25%, respectively (Figure 2).
Conclusions: Collectively, the results of this study present a promising strategy for identification of potential PTOA related therapeutic targets. This system permits the rapid identification of compounds that may act to interfere with the chondrocyte's response to injury, both in regard to the early cell death and the later matrix degradation that occurs. With the successful follow up of positive hits with additional molecular pathway assays and an in vivo injury animal model, this new testing platform has the potential to identify new and early therapy for this common clinical problem of trauma-related osteoarthritis,

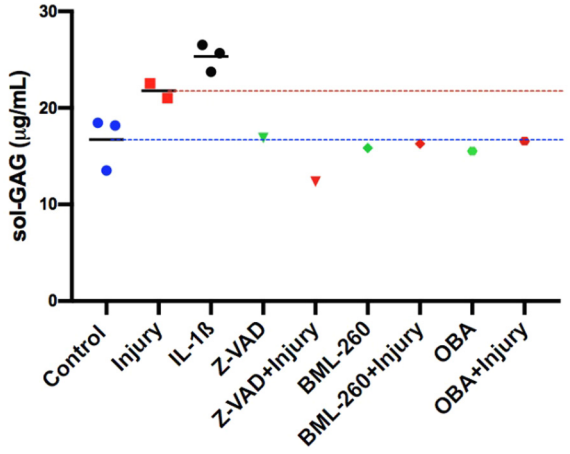

Figure 2. Example of GAG concentrations released to media 48 hour post injury. The dotted red and blue lines denote the average GAG concentrations of the injured/non-treated group and the naive control group, respectively. Two secondary screenings were performed with these compounds at a series of dilutions and time and for simplicity a final repeat primary screening is shown.

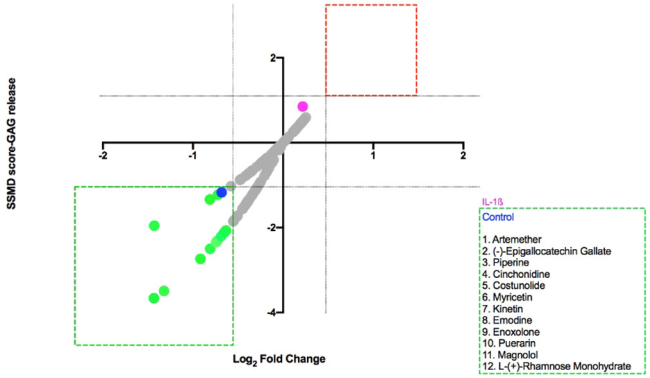

Figure 1. Primary screening results of the Natural Compound Library (Selleckchem), with respect to s-GAG release at 48 hours post injury. A Dual-Flashlight plot was utilized with gating parameters 1 ≥ SSMD ≥ 1 and 0.5 ≥ Fold Change ≥ 0.5 to determine potential therapeutic "hits" that are advanced to secondary screening.

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DO CORTICOSTEROIDS OR HYALURONIC ACID INTRA-ARTICULAR INJECTIONS IMPACT THE RISK OF TOTAL KNEE REPLACEMENT? REAL-LIFE DATA FROM THE KHOALA COHORT

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Purpose: Long-term structural outcomes of corticosteroids (CS) or hyaluronic acid (HA) intra-articular injections in knee osteoarthritis (KOA) are unclear. Whether HA injections delay the need for total knee replacement (TKR) remains controversial, and an increased risk of

cartilage damage has been reported with repeated CS injections. We conducted this study to compare, in real-life setting, the risk of TKR in patients receiving CS or HA vs. in those who did not receive intra-articular injections.

Methods: Khoala cohort is a French nationwide population-based cohort of 878 patients with symptomatic hip or knee OA (ACR criteria), aged 40-75 years. This study included patients with baseline KOA only. Patients were followed annually by self-reported questionnaires and by clinical examination and radiography at baseline (year 0), years 3 and 5. The risk of incident TKR was compared between patients who had never received intra-articular injections vs. patients who received at least 1 CS or HA injection during follow-up. We used a marginal structural model with inverse probability of treatment weighting to determine the causal relationship between treatment and the risk of incident TKR in the treated knee during the 5-years follow-up. This model allows for the adjustment for time-varying confounding factors (i.e. pain, function and mental health scores) in addition to constant confounders (i.e. baseline age, sex, BMI, Kellgren-Lawrence grade).

Results: This study involved 656 patients (mean age 62.2 ± 8.5 years; 70.3% females) of which 91 (13.9%) underwent TKR during follow-up. CS or HA injections were performed in 143 (21.8%) and 191 (29.1%) patients, respectively, and 92 (14.0%) received both treatments. The 5-year relative risk of incident TKR in treated vs. untreated knee was 0.96 (95%CI 0.35 to 2.66; p=0.94) CS-treated knees and 0.38 (95%CI 0.15 to 1.03; p=0.06) in HA-treated knees.

Conclusions: In this real-life study, CS injections for symptomatic KOA did not increase the 5-year risk of incident TKR. There was a non-significant trend for a reduced risk of TKR in HA-treated knees.

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VITAMIN K INHIBITOR USAGE IS ASSOCIATED WITH INCREASED INCIDENCE AND PROGRESSION OF KNEE AND HIP OSTEOARTHRITIS

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Purpose: Several lines of evidence implicate vitamin K (vitK) in osteoarthritis. A number of observational studies reported an association between Vitamin K status and prevalence and incidence of osteoarthritis. Furthermore, vitK dependent proteins, with the most prominent one being Matrix Gla protein (MGP), are abundantly found in cartilage and bone. MGP is an essential regulator of chondrogenesis and inhibitor of cartilage calcification. Unactive MGP is elevated in human arthritic cartilage, while active MGP is more abundant in healthy cartilage. Activation of MGP is fully dependent on Vitamin K. Recently, a large scale genetic study have also identified MGP to be causally involved in osteoarthritis. A relatively modestly sized ancillary study to a vitamin K supplementation trial (primary endpoint vascular calcification) observed a beneficial effect on OA-prevalence in those individuals that were Vitamin K deficient. All these studies link vitamin K status to osteoarthritis. We therefore aimed at investigating the effect of acenocoumarol usage, a vitamin K inhibitor prescribed as anti-coagulant, on progression of radiographic osteoarthritis.

Methods: We used data from in total 5,218 elderly participants that were not using acenocoumarol at baseline, from two prospective population based cohorts within the Rotterdam Study (RS). Radiographs of the knees and hips were obtained at baseline and after 10 years of follow up. Any increase in KL-score was defined as overall progression (incidence and progression). Information on acenocoumarol usage during follow-up was collected from computerised pharmacy databases. The risk of osteoarthritis progression in acenocoumarol users was compared to that of non-users. In addition, we separately examined participants that used acenocoumarol more then 100 days. Adjusted logistic regression analyses with generalized estimating equations were used to calculate Odds Ratios and 95% CIs for each joint group separately after adjusting for confounding variables. Confounding variables included, age, sex, BMI, follow-up time, KL-score at baseline. In an additional analysis, possible comorbidity confounders were added: hypertension, diabetes mellitus, HDL/total cholesterol ratio, education, peripheral artery disease and smoking.

Results: The number of joints that showed progression/incidence was 274(4%) and 424(7%) for hip and knee respectively in RS1, while 64(2%) and 145(5%) progression/incidence cases were present in RS2. In both cohorts, acenocoumarol usage was associated with a more then 2 times higher risk for OA progression, both for the knee and the hip (Table 1). Meta-analysis of the results showed that acenocoumarol use was robustly associated with a more than 2 times higher risk for radiographic osteoarthritis progression in the knee (OR 2.03 95%CI: 1.52 - 2.71, p<0.001), see table 1) and hip (2.86 (2.05 - 3.99; p<0.001), see table 1). Consistent effects were seen in both men and women. Additional adjustment for potential comorbidity and lifestyle confounders did not affect the risk estimates.

Conclusions: Acenocoumarol usage was associated with a more then 2 times higher risk of osteoarthritis progression in both hip and knee joints. Vitamin K inhibitors, possibly through the inhibition of Matrix Gla Protein function, may modify the development and progression of osteoarthritis

		Meta-analysis OA progression and acenocoumarol use in RS1 + RS2			OA progression and acenocoumarol use in RS1			OA progression and acenocoumarol use in RS2		
	Acenoc. Use	No. of joints	progression, nr(%)	OR (95% CI)	No. of joints	progression, nr(%)	OR (95% CI)	No. of joints	progression, nr(%)	OR (95% CI)
Knee OA progression	All use									
	no	8188	481 (5.9)	1	5550	352 (6.3)	1	2638	129 (4.9)	1
	yes	657	88 (13)	2.03 (1.52 - 2.71)	532	72 (14)	1.99 (1.44 - 2.74)	125	16 (13)	2.33 (1.17 - 4.62)
	>=100 days use									
	no	8314	496 (6)	1	5641	364 (6.5)	1	2673	132 (4.9)	1
	yes	531	73 (14)	2.13 (1.55 - 2.93)	441	60 (14)	2.08 (1.45 - 2.97)	90	13 (14)	2.52 (1.28 - 4.97)
Hip OA progression	All use									
	no	8464	262 (3.1)	1	5821	212 (3.6)	1	2643	50 (1.9)	1
	yes	641	76 (12)	2.86 (2.05 - 3.99)	510	62 (12)	2.57 (1.8 - 3.67)	131	14 (11)	6.17 (2.44 - 15.6)
	>=100 days use									
	no	8574	277 (3.2)	1	5897	224 (3.8)	1	2677	53 (2)	1
	yes	531	61 (11)	2.5 (1.72 - 3.63)	434	50 (12)	2.27 (1.53 - 3.34)	97	11 (11)	5.58 (1.77 - 17.6)

Table 1: Increased risk for progression of knee or hip OA in participants using acenocoumarol vs those that did not use. Model included the following covariates: Age, BMI, gender, KL-score at baseline, Follow-up time. Additional adjustment for comorbidity, including cholesterol levels, diabetes, cardiovascular disease did not affect the risk estimates.

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PREPARATION OF A POLYMERIC INTRA-ARTICULAR PPAR DELTAANTAGONIST DELIVERY SYSTEM

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Purpose: Recently, PPARδ inhibition has been studied for its potential role in the breakdown of cartilage in Osteoarthritis (OA). We demonstrated that genetic inhibition of PPARδ attenuated progression of OA in a surgical mouse model (Ratneswaran et al., 2015). Due to the hydrophobicity of pharmacological PPARδ inhibitors, and systemic side effects, delivery of these inhibitors has proven challenging. This research studies the preparation of a polymeric drug delivery system for the PPARδ inhibitor, GSK 3787. The delivery system is designed to encapsulate drugs, be injectable into the joint, and provide a prolonged release of medication.

Methods: Using a poly(ester amide) particle delivery system that had been previously researched for the encapsulation of celecoxib, a PPARδ inhibitor delivery system was prepared. Polymer particles were made through an emulsification evaporation technique with GSK 3787 encapsulated. Particles were characterized physiochemically, including their morphology, size, drug loading content, in vitro release profiles, and mechanical properties. Furthermore, in vitro toxicity was assessed by MTT assay on primary immature murine articular chondrocyte (IMAC) cell cultures, as well as the ATDC5 cell line. Cells were imaged with confocal microscopy to establish their interaction with particles, and qPCR was used to determine the molecular effects of the released GSK 3787 in cells.

Results: Preparation of the particle delivery system yielded particles that were found to be 500 nM in size, with smooth and consistent morphology. Particles were found to have a drug loading percentage of 8.6%, and their encapsulation efficiency was 97%. In vitro release data shows a slow and prolonged release of the loaded drugs, with no burst release present. Cell toxicity studies on IMAC cells showed no toxicity caused by the particles, at a wide range of concentrations. When viewed with confocal microscopy, it was determined that particles do not enter cells, but aggregate and interact with the cell surfaces; cells retain their shape and attachment to the cell culture dishes and appear to continue their growth.

Conclusions: The development of a PPARδ intra-articular delivery system was successful. The system was prepared and characterized through in vitro studies. Further work remains to test the in vivo response of the delivery system to injection in an animal model. This work serves to be the first system for the delivery of a potential PPARδ inhibitor in the treatment of OA.