the effect is limited by a narrow therapeutic window for Treg selectivity. Furthermore, the short half-life of IL-2 necessitates frequent administration. NKTR-358 is a polyethylene glycol (PEG) conjugate of recombinant human IL-2 (aldesleukin sequence) and is differentiated from native IL-2 by its altered binding to the IL-2 receptor and prolonged biological activity. NKTR-358 resulted in marked and selective stimulation of Tregs when administered as a single SC injection to healthy volunteers.

Objectives: This multiple ascending dose study assessed the safety, tolerability, pharmacokinetics (PK), and immune effects of NKTR-358 in patients with SLE after repeated administration of SC doses. The time course and extent of changes in numbers and percentages of Tregs, conventional CD4+ and CD8+ T cells, NK cells, and cytokine levels in peripheral blood were investigated.

Methods: In this double-blind, multiple ascending dose study, patients with mild to moderate SLE received 3 SC doses q2w in 4 cohorts ranging from 3.0 to 24.0 µg/kg (9 active:3 placebo per cohort); patients were followed for a total of 79 days.

Results: There were no dose-limiting toxicities, deaths, or clinically significant abnormalities in either vital signs or electrocardiograms. Adverse events attributed to NKTR-358 were primarily limited to mild (grade 1) injection site reactions. At the highest dose, one subject had transient and mild (grade 1) symptoms of a flu-like syndrome after administration, without associated elevated cytokine levels, and another subject had dosing stopped due to elevated eosinophil levels. No other individual at any dose level had systemic signs or symptoms known to be associated with IL-2 therapy. No anti-drug antibodies were detected. NKTR-358 demonstrated dose-proportional PK with repeated dosing; plasma levels peaked 3-6 days post-dose and declined with a terminal half-life of ~10-13 days.

The primary and consistent effect of NKTR-358 was seen on Tregs. In the four dose cohorts, dose-dependent and sustained increases in absolute numbers and percentages of circulating CD4+FoxP3+CD25^{bright} Tregs were observed. Treg levels remained elevated throughout the dosing period, peaking at Day 10 after the first administration of NKTR-358 and returning to baseline ~20-30 days following last administration. At 24.0 µg/kg, the mean peak increase in numbers of CD25^{bright} Tregs was 11-fold above baseline. In addition, there was an increase in Treg activation markers at doses ≥12.0 µg/kg. In contrast to effects on Tregs, no changes in percentages or numbers of conventional CD4+ or CD8+ T cells were observed at any dose tested. At the highest dose, there were low-level increases in the percentages and numbers of NK cells. Overall, NKTR-358 selectively induced Tregs, evidenced by a 12-fold increase in the mean peak Treg:CD8 ratio over baseline in the 24.0 µg/kg group.

Conclusion: NKTR-358, an IL-2 conjugate Treg stimulator, was well tolerated when repeatedly administered (q2w) at doses up to 24 µg/kg. Its administration led to marked, selective, prolonged, and dose-dependent increases in circulating CD25^{bright} Tregs. This clinical study in SLE patients extends the previous results in healthy volunteers and provides strong support for continued testing of NKTR-358 as a new therapeutic in SLE and other inflammatory diseases.

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OA, aetiology, pathology and animal models

THU0055

ANTI-DEGRADATIVE AND PRO-CHONDROGENIC PROPERTIES OF LIRAGLUTIDE, A GLUCAGON-LIKE-PEPTIDE 1 RECEPTOR AGONIST: EVIDENCE FROM PRECLINICAL STUDIES AND IMPLICATION FOR OSTEOARTHRITIS

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Background: Osteoarthritis (OA) is a degenerative joint disease affecting millions of individuals worldwide. Its development has been reported to be associated with cartilage degradation and inflammatory responses leading to pain, swelling and reduced function. Although OA is a disorder of the whole joint, the progressive destruction of cartilage extracellular matrix is considered as its hallmark. To date, approved OA treatments are only symptomatic. Therefore, there is an urgent need to explore disease-modifying OA drugs (DMOADs) that can mitigate, stop, or even reverse the development of OA.

Objectives: In this context, the objective of this study was to assess the effect of liraglutide, a Glucagon-Like-Peptide 1 Receptor (GLP-1R) agonist approved for type 2 diabetes, on chondrogenesis, catabolism/inflammation and cartilage protection in *in vitro* and *in vivo* preclinical models of OA.

Methods: The capacity of liraglutide to induce chondrogenesis was evaluated using primary human mesenchymal stem cells (hMSCs). Alcian blue staining was used to assess differentiation of hMSC into chondrocyte spheroids. IL-1β-stimulated mouse articular chondrocytes were treated with different concentrations of liraglutide for 24h. Production of matrix metalloproteinase MMP-13, prostaglandin E2 (PGE2) and nitrite was measured by ELISA and Griess reaction, respectively. Exendin 9-39, a GLP-1R antagonist, was used to confirm target engagement in the *in vitro* experiments. Intra-articular (IA) injections of liraglutide or vehicle were performed in the type II collagenase rat model. Histopathological analyses (OARSI scores¹) were conducted blindly by one investigator.

Results: Liraglutide induced the differentiation of hMSCs into chondrocytes. Indeed, 21 days after differentiation initiation, 5/6 and 4/6 alcian-blue positive spheroids were observed for 10 and 100nM liraglutide, respectively, versus 0/6 for vehicle. Liraglutide significantly reduced dose-dependently the IL-1β-induced production of PGE2 (5808±178 for vehicle vs 4560±140, 2933±171 and 2365±85 pg/ml for liraglutide 10, 100 and 500nM, respectively, p≤0.001), nitrite (24.9±0.4 for vehicle vs 20.9±1.5, 19.1±0.9 and 16.5±0.5 µM for liraglutide 10, 100 and 500nM, respectively, p≤0.001) and MMP-13 (686±9 for vehicle vs 553±3, 402±5 and 297±8 pg/ml for liraglutide 10, 100 and 500nM, respectively, p≤0.001) in murine chondrocytes. Effects of liraglutide were GLP-1R dependent since exendin 9-39 significantly counteracted both chondrogenesis and inflammation/catabolism markers expression. Histological assessment of rat collagenase-injected knee joint revealed a significant (p≤0.05) decrease of the total joint score in the IA Liraglutide treated group (8±4) compared to vehicle (11±4).

Conclusion: Liraglutide induced chondrogenesis, decreased metalloproteinase and inflammatory mediators production by chondrocytes and protected cartilage in *in vitro* and *in vivo* preclinical OA models, opening the way for repositioning this drug as a potential DMOAD.

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THU0056

SM04755, A POTENTIAL DISEASE-MODIFYING TREATMENT FOR TENDINOPATHY, MODULATES THE WNT PATHWAY VIA INHIBITION OF CLKS AND DYRK1A

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Background: Tendinopathy is an inflammatory and degenerative disorder of tendons caused by injuries and/or overuse. Left untreated, tendinopathy can lead to pain and rupture. Current therapeutic options only treat symptoms. Stem cell- and growth factor-based treatments are under investigation but have not

established safety or efficacy, leaving considerable unmet need. The Wnt pathway is upregulated in chronic tendinopathy, affecting inflammation and tenocyte differentiation. SM04755, a novel, topical, small-molecule Wnt pathway inhibitor, has previously been shown to inhibit inflammation, protect tenocytes, and increase tenocyte differentiation in nonclinical models.¹

Objectives: To identify molecular targets of SM04755 and its associated mechanism of action.

Methods: Wnt pathway inhibition was measured using a cell-based luciferase reporter assay controlled by a β -catenin/TCF-responsive promoter in SW480 colon cancer cells. A kinome screen (318 kinases) and kinase assays were performed. Effects of SM04755 on phosphorylation of proteins, including serine/arginine-rich splicing factor (SRSF) proteins in rat tendon-derived stem cells (rTDCs) and peripheral blood mononuclear cells (PBMCs), were measured using western blot. siRNA-mediated knockdown of CDC-like kinases (CLKs) and dual-specificity tyrosine kinase (DYRK1A) were performed in human mesenchymal stem cells (hMSCs), rTDCs, and rat tenocytes. Effects of SM04755 and siRNA knockdowns on Wnt pathway gene expression and catabolic enzymes (MMPs) were measured using qPCR. SM04755 and siRNA effects on tenocyte marker expression were assessed by qPCR and immunostaining. Effects of SM04755 on LPS-induced expression of inflammatory cytokines in PBMCs were measured by MSD-based ELISA. Statistical analyses used one-way ANOVA for multiple group comparisons and t-tests for comparison between two groups.

Results: SM04755 was a potent inhibitor ($EC_{50}=156$ nM) of Wnt signaling. Biochemical assays identified CLKs and DYRK1A as molecular targets of SM04755. SM04755 potentially inhibited CLK-mediated phosphorylation of SRSF proteins compared with DMSO controls. Knockdowns of CLKs and DYRK1A led to inhibition of Wnt pathway genes (*AXIN2*, *LEF1*, *TCF4*, *TCF7*, etc.) compared with siRNA controls (siCtrl). CLK1, 2, and 4 and DYRK1A knockdowns also induced expression of tenocyte markers in rTDCs and inhibited IL-1 β -induced expression of catabolic enzymes (MMP1, 3, 9, 13) in tenocytes compared with siCtrl. SM04755 treatment of LPS-stimulated PBMCs resulted in reduced phosphorylation of NF- κ B and STAT3 and inhibited production of inflammatory cytokines compared with DMSO.

Conclusion: SM04755 inhibited CLKs and DYRK1A, which led to Wnt pathway modulation. Knockdowns of CLKs and DYRK1A, compared with control siRNA, induced tenocyte differentiation and reduced tendon-destroying proteases in tenocytes. This supports the potential disease modification of tendinopathy with SM04755. Furthermore, the anti-inflammatory effects of SM04755 are mechanistically supported by the decreased phosphorylation of STAT3 and NF- κ B. These data support that SM04755, as a single agent, may potentially improve symptoms and provide disease modification in tendinopathy. Human tendinopathy trials are planned.

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THU0057

A NEO-EPITOPE FRAGMENT OF CARTILAGE DEGRADATION GENERATED FROM TYPE II COLLAGEN PROCESSING: A NOVEL SERUM BIOMARKER TO ACCESS TYPE II COLLAGEN DEGRADATION IN JOINT DEGENERATIVE DISEASES

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Background: Altered extracellular matrix (ECM) remodelling is an important part of the pathology seen in joint degenerative diseases. Type II collagen is the most abundant ECM protein in the cartilage and provides the tissue with essential tensile strength in order to withstand high compressive loading. During cartilage erosion, type II collagen is cleaved by matrix metalloproteinases (MMPs) which generates new protein fragments called neo-epitopes. These fragments are

released into circulation and may potentially serve as biomarkers by indicating the degree of cartilage destruction.

Objectives: The aim of this study is to develop a highly specific immunoassay targeting a neo-epitope fragment of type II collagen cleaved, named T2CM. Moreover, we investigated the assays potential to evaluate type II collagen degradation in an ex vivo bovine full-depth cartilage explants model (BEX) with catabolic treatment and in healthy controls and osteoarthritis (OA) patients.

Methods: A monoclonal antibody was raised in mouse against the C-terminus from protease cleavage site of type II collagen and a direct competitive ELISA was developed and technically validated. The assay specificity was evaluated for the standard peptide excluding cross-reactivity with elongated and truncated peptides, and a non-sense coating peptide. Human OA cartilage was cleaved with MMP-1, -2, -9 and -13 and measured with the T2CM-assay to investigate which MMPs generated the neo-epitope. T2CM levels were measured in supernatant from BEX explants cultured for 21 days in serum free DMEM/F12 medium with six different doses of OSM+TNF- α (O+T) treatment (20/10, 20/20, 20/40, 10/10, 10/20, 10/40 ng/mL) including a control group without (w/o) treatment. The supernatant was harvested 3 times weekly and replaced with new culture medium with O+T treatment. Biomarker results were confirmed by western blot, where T2CM was measured in supernatant from explants with O+T treatment 20/20 ng/mL and 20/40 ng/mL harvested on day 14 and day 21. To confirm the preclinical data, serum samples from 23 healthy controls (age range from 44-59 years with mean $51.4 \pm SD 5.1$, gender distribution was 56% female and 44% male, and 100% Caucasian) and 23 OA patients (age range from 41-77 years with mean $57.7 \pm SD 13.7$, gender distribution was 61% female and 39% male, and 100% Caucasian) were measured by T2CM.

Results: A technically robust and T2CM-specific assay was developed. The assay linearity and spike-recovery were accepted with percentage of 99.69% and 93.15%. The assay showed no cross-reaction with the elongated, truncated or non-sense coating peptide. In addition, it was demonstrated that the T2CM neo-epitope was derived from MMP-1 and MMP-13 cleavage of type II collagen. O+T treatment induced the T2CM release in BEX compared to the untreated (Figure 1-2). Moreover, the western blot confirmed the T2CM results by the presence of human OA bands on day 21 from O+T treated explant compared to day 14 where no bands appeared. T2CM showed to be significantly elevated in patients with OA compared to controls ($p=0.036$; mean 3.262 ng/mL $\pm SD 1.065$ vs 2.698 ng/mL $\pm SD 1.118$).

Conclusion: The newly developed assay was specific for the T2CM neo-epitope and was determined to be generated by MMP-1 and MMP-13. Additionally, the assay detected elevated levels of T2CM in supernatant from explants treated with O+T after 19 days of treatment compared to untreated. This was further confirmed in human OA patients, where the level of T2CM was elevated compared to healthy controls. This suggests that T2CM may have potential as biomarker for type II collagen degradation. Future preclinical and clinical studies are needed to validate these findings.

Figure 1-2. T2CM measurements in BEX model. OSM + TNF- α (O+T) ng/mL.

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