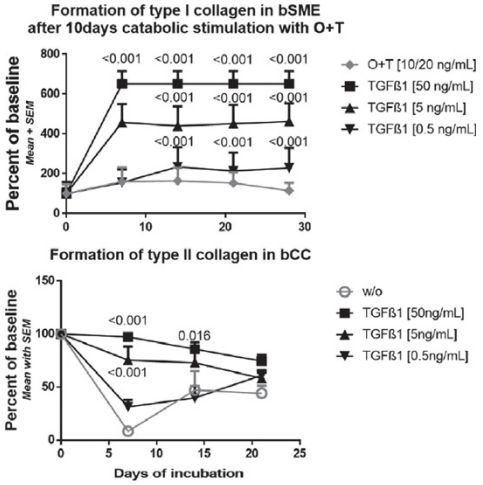
and PRO-C2), aggrecanase degraded aggrecan (AGNxI) and MMP degraded aggrecan (exFFGV). C1M and C3M are synovial membrane biomarkers and C2M, AGNxI and exFFGV are cartilage biomarkers.

Results: Explants were viable throughout the experiments, albeit the bSME lost some viability with time. bSME treated with O+T showed increased C1M and C3M (>400% and >200%) from day 10, compared to w/o, whereas in bCC O+T increased C1M from day 21 and C3M from day 14 (>800%, >1900%). O+T treatment increased C2M was increased from day 21 (>400% and >1000%) in both BEX and bCC. The release was blocked by the generic MMP inhibitor GM6001 which also decreased the C1M and C3M compared to w/o. O+T treatment increased AGNxI at day 7 and 10 (>600%) and exFFGV from day 21 (>650%) in both BEX and bCC. In bSME, TGF-b1 continuously and dose-dependently increased P1NP from day 7 compared to w/o (250%). O+T pre-treatment for 10 days followed by TGF-b1 stimulation increased P1NP after 7 days of TNF-b1 treatment (150%, figure). IGF-1 did not affect the P1NP level at any time point in bSME. In bCC both TGF-b1 (dose-dependently) and IGF-1 sustained the PRO-C2 level at the level of baseline throughout the study periods (figure), whereas O+T decreased PRO-C2 compared to with w/o. The PRO-C2 level in BEX with TGF-b1 was unaltered compared to w/o.



Conclusions: We here show that bovine synovium can be anaobolic and catabolic stimulated, both alone and in co-culture with cartilage. Anabolic stimulation was achieved with TGF-b1 in both bSME and bCC, while IGF-1 only showed an anabolic effect in the bCC. Previous human explant models using human tissuehave lacked the anabolic capacity. These translational explants models may be applied in the early development of anabolic drugs for cartilage degenerative diseases.

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FRI0004 14-3-3ETA AS A NOVEL INVADOSOME REGULATORY MOLECULE IN RHEUMATOID ARTHRITIS

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Background: Progressive cartilage destruction, mostly mediated by invasive fibroblast-like synoviocytes (FLS), is a central feature in the pathogenesis of rheumatoid arthritis (RA). We have reported that the ability of arthritic FLS to degrade the extracellular matrix depends on the formation of actin-rich plasma membrane invadosomal structures detected in cells strategically located at the cartilage-synovial membrane interface. Interference with the formation of invadosomes in RA FLS impeded matrix degradation in vitro and cartilage degradation in a model of collagen-induced arthritis, suggesting that invadosomes are important physiological structures involved in cartilage destruction.

The chaperonin molecule 14–3-3η has been detected in the joints of patients with early and established RA and that its concentration in both serum and synovial fluid correlated with elevated expression of extracellular matrix (ECM) degrading enzymes and erosive damage. Extracellular 14–3-3η has therefore been proposed to be a novel biomarker for joint damage and a potential drug target for the personalized treatment of connective tissue-associated diseases but the direct relationship between 14–3-3η and joint damage remains a key area of research.

Objectives: To evaluate the role of 14–3-3η in the ability of synoviocytes to degrade the ECM.

Methods: mRNA from primary synoviocytes of healthy individuals (N=3) and RA patients (N=5) was extracted and the relative level of 14–3-3 isoforms and MMP gene expression was determined by qPCR. The ability of the synovial cell lines to degrade ECM was assessed by in situ invadosome assays using fluorescent cross-linked gelatine. Confocal microscopy was used to determine the cellular localization of 14–3-3η.

Results: We found a significant increase in 14–3-3η, MMP1 and MMP3 mRNA levels in synoviocytes from rheumatoid arthritis patients compared to cells from non-arthritis individuals. A strong correlation between 14–3-3η expression levels and the ability of synoviocyte cell lines to form invadosomes was observed (r2=0.8299). Knockdown of 14–3-3η decreased the ability of arthritic synoviocytes to form invadosomes indicating a role of 14–3-3η in extracellular matrix degrading ability. Confocal microscopy revealed that 14–3-3η staining was mostly found as small punctated structures in the cytoplasm and at the cell periphery of arthritic synoviocytes where they colocalized with leading edge F-actin and discrete patches of the exocyst component, Exo70.

Conclusions: The finding of the role of 14–3-3η in invadosome formation points to a previously unappreciated facet of how 14–3-3η influences joint ECM remodelling and reinforces its role as a marker of RA progression and joint damage. How 14–3-3η is involved in the regulation of MMP production/secretion and the possible role it plays in remodelling of actin-rich subcellular structures is the subject of ongoing studies.

Disclosure of Interest: C. Lalanne: None declared, R. Lavoie: None declared, M. Charbonneau: None declared, J. Savill Employee of: Augurex Life Sciences Corp., A. Marotta Employee of: Augurex Life Sciences Corp., C. Dubois: None declared

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FRI0005 TARGETING CARTILAGE AGING AS OSTEOARTHRITIS THERAPEUTICS BY DRUG REPURPOSING

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Background: Effective treatments for Osteoarthritis (OA) are not available. In aging-related diseases, including OA, failure of cellular homeostasis mechanisms, such as autophagy can cause extracellular matrix destruction and cell death (1). With aging, chondrocyte function is diminished, contributing to a cellular senescence phenotype often observed in OA chondrocytes. In addition, a defect in autophagy is observed in both aging and cartilage degeneration (2,3).

Objectives: The objective of this study was to identify anti-senescence and pro-autophagy molecules by a cell-based high-throughput screening (HTS) in human chondrocytes.

Methods: To induce cellular senescence or reduced autophagy, immortalized human chondrocytes (TC28a2) were seeded (2500 cells/well) in 384 well plates, and treated with IL-6 (20 ng/ml) for 72 or 18 hours, respectively. Then, chondrocytes were incubated with Prestwick Chemical Library (1120 approved drugs with chemical and pharmacological diversity, as well as bioavailability and safety in humans) at 10 μM for 72 hours. To identify anti-senescence hits, nuclei was stained with Hoechst 33342 (2,5μg/ml), while β–galactosidase subcellular structures was stained by using Imagene Green C12FDG substrate (30 μM). To evaluate autophagic flux, a reporter cell line was generated by retrovirus transfection of pBABE-mCherry-EGFP-LC3 plasmid in TC28a2 chondrocytes. Plates were imaged by using Operetta® High Content Screening (HCS) system in non-confocal mode using the 20x WD objective. For each well, 4 fields and 4 planes of bright field, Hoechst and fluorescein channels were obtained. Relative intensity of C12FDG in cytoplasm and number of autophagosomes/autolysosomes per area of cytoplasm were determined to quantitate β–galactosidase activity and autophagy flux respectively. Compound validation was performed in TC28a2 chondrocytes and in primary human chondrocytes by evaluating cell senescence, autophagy pathway and cell death by apoptosis.

Results: A primary screening was performed to identify anti-senescence compounds by measurement of senescence-associated β-galactosidase activity. 283 compounds with anti-senescence effects were identified. The anti-senescence hits were analyzed by monitorizing autophagic flux. 29 compounds with both anti-senescence and pro-autophagy effects were selected. Then, one compound was selected for further validation. The compound reduced chondrocyte senescence, increased autophagy ($p<0.0001$) and protected against inflammation and cell death by apoptosis in human chondrocytes ($p<0.05$) in response to IL-6. Interestingly, this protective effect was partially mediated by mTOR inhibition, a proposed mechanism to prevent cartilage aging.

Conclusions: Our screening methodology provides a unique opportunity to repurpose drugs and mechanisms to prevent cartilage aging. Autophagy activation and protection against senescence in chondrocytes may provide benefits for delaying cartilage degeneration.

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FRI0006 PROTEIN CITRULLINATIONS BY PAD ENZYMES PROMOTE DENDRITIC CELL TRANSDIFFERENTIATION INTO OSTEOCLAST AND GENERATE TARGETS FOR RA-SPECIFIC ANTIBODIES

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Background: Immature dendritic cells (DCs) are able to trans-differentiate into osteoclasts (OCs) although the mechanisms regulating this process are little understood. We have recently described an important role for protein citrulliantion and peptidylarginine deiminase (PAD) enzyme activity in the regulation of OC development (1).
Objectives: We studied the molecular bases of DC-OC trans-differentiation and aimed at understanding the role of protein citrullination in this process.
Methods: Monocyte-derived DCs and peripheral blood CD1c+ DCs were cultured in the presence of osteoclastogenic cytokines. Polyclonal ACPAs were isolated from the serum of RA patients and applied in OC cultures. DC and OC differentiation was analyzed *in vitro* using gene expression analyses, flow cytometry-based methods, DC-T cell co-culture experiments, tartrate-resistant acid phosphatase stainings and osteolysis assays. Protein citrullination was monitored with the help of mass spectrometry. PAD activity was measured by ELISA.
Results: Different DC types showed different capacities to develop into OCs. OC-prone DCs were characterized by little immunogenicity and their development was potentiated by the increase of lactic acid, a side product of glycolytic metabolism. The more immunogenic DC types, characterized by prominent ability to migrate towards secondary lymphoid tissues and trigger T cell activation, showed a limited capacity to develop into OCs (2). The differentiation switch towards the OC lineage was associated with increased activity of the Protein Arginine Deiminase (PAD) enzymes and with higher level of protein citrullination in DCs. The PAD inhibitor Cl-Amidine efficiently interfered with OC development form DC precursors. In addition, the deposition of citrullinated proteins on the cell surface made the cells sensitive for anti-citrullinated protein autoantibodies, which could further stimulate DC-OC trans-differentiation through inducing the cytokine IL-8.
Conclusions: Our results indicated that DCs are heterogenic in their ability to form OCs and lineages for immunostimulatory and OC-prone DCs might separate early during DC differentiation. Plasticity towards OC differentiation might be influenced by the metabolic environment and the upregulation of PAD activity in DCs.
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FRI0007 METHYL GALLATE INHIBITS OSTEOCLAST FORMATION AND FUNCTION THROUGH SUPPRESSING THE AKT AND BTK-PLCγ2-CA2+ SIGNALING, AND PREVENTS LPS-INDUCED BONE LOSS

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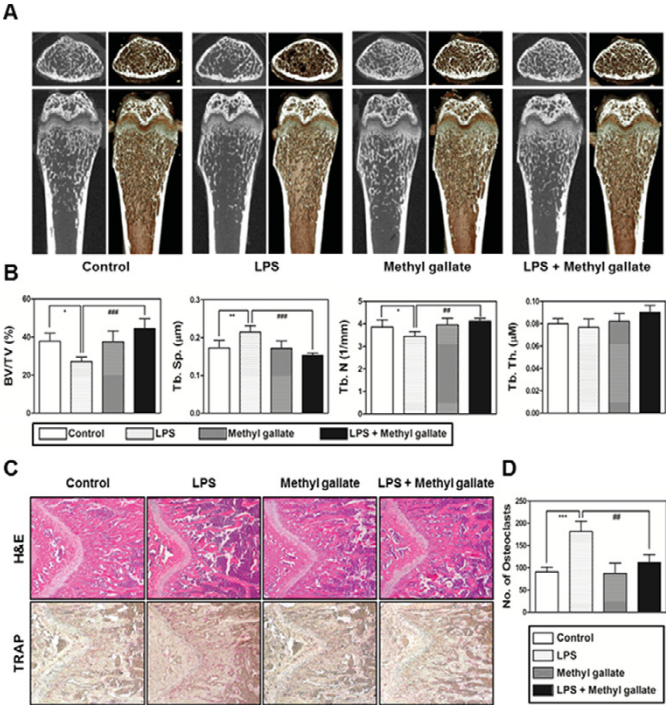
Background: Methyl gallate, a plant-derived phenolic constituent has been known to possess numerous pharmacological features against inflammation, oxidation, and cancer. But so far, there have been no evidences to describe relationship between methyl gallate and bone metabolism.
Objectives: In order to propose a promising candidate for osteoporosis, we performed experiments in this study by using methyl gallate.
Methods: we performed screening of methyl gallate utilizing TRAP staining and revealed intracellular mechanisms responsible for methyl gallate-mediated regulation of osteoclastogenesis through western blotting and quantitative RT-PCR. Also, we assessed the role of methyl gallate on characteristics of mature osteoclasts. we used LPS-induced bone loss mice as a model of osteoporosis and analyzed using micro-CT system and the right femurs were stained with TRAP and H&E.
Results: we observed that methyl gallate significantly suppressed osteoclast formation through Akt and Btk-PLCγ2-Ca^{2+} signaling. The blockade of these pathways was reconfirmed through transduction of CA-Akt retrovirus and evaluation of Ca^{2+} influx intensity stained with Fluo-3/AM. Indeed, methyl gallate down-regulated the formation of actin ring-positive osteoclasts and resorption pit areas. In agreement with in vitro results, we found that the administration of methyl gallate restored osteoporotic phenotype stimulated by acute systemic injection of LPS in vivo through micro-CT and histology.



Conclusions: Consequently, the overall data strongly indicated that methyl gallate could be a useful substance for development of plant-based anti-osteoporotic agent.
Disclosure of Interest: None declared
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FRI0008 EFFECTS OF THE HUMAN IL4-10 FUSION PROTEIN IN THE CANINE GROOVE MODEL OF OSTEOARTHRITIS

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Background: Ideally a disease modifying osteoarthritis drug (DMOAD) combines treatment for pain, tissue damage and inflammation, all in one molecule. Intra-articular application of a DMOAD brings additional value to treatment for two reasons, (i) lower risk of systemic side effects and (ii) higher drug concentration and potentially improved penetration of non-vascularized articular cartilage. Interleukin-4 (IL-4) and Interleukin-10 (IL-10) have been shown to prevent joint degeneration and can work synergistically[1].
Objectives: This study evaluates the DMOAD activity of repetitive intra-articular injections with a human fusion protein of IL-4 and IL-10 (hIL4–10FP) in the canine Groove model of osteoarthritis (OA).
Methods: In 8 dogs joint degeneration was induced according to the Groove model. Six weeks after surgery dogs were treated with ten weekly intra-articular injections of either hIL4–10FP (n=4) or PBS (n=4). Subsequently, dogs were euthanized and cartilage and synovium were harvested. Cartilage damage and synovial inflammation were macroscopically evaluated. Proteoglycan release and content were determined ex vivo by staining of glycosaminoglycans (GAGs) with Alcian Blue. Proteoglycan synthesis was measured by $^{35}SO_4^{2-}$ incorporation and precipitation with cetylpyridium chloride and liquid scintillation analysis of $^{35}SO_4^{2-}$-labeled GAGs. Potential antibody formation against hIL4–10FP was evaluated with ELISA and a cell based assay. Immunohistochemistry of CD79α and CD10 was used to evaluate the presence of B-cells in synovium.
Results: Affected knees of PBS treated dogs showed enhanced macroscopic cartilage damage compared to their controls (0.31 vs 2.44, p=0.068). Also differences in proteoglycan release, content and synthesis indicated a degenerative state in the affected knees.
Unexpectedly, enhanced synovial inflammation was observed in hIL4–10FP treated joints compared to PBS treated joints, demonstrated by enhanced macroscopic (3.5 vs 2.3 out of 5) and histologic (2.5 vs 1.8 out of 6) scores. CD79α and CD10 showed enhanced expression in synovium of the hIL4–10FP group compared to the PBS group, although not statistically significant (fig 1). Additional analyzes showed that hIL4–10FP was immunogenic in dogs after multiple injections. Formation of neutralizing antibodies was shown in a cell based assay where the activity of hIL4–10FP was inhibited in the presence of serum of hIL4–10FP treated dogs (fig 2). Despite the enhanced inflammatory response