confinement. Further safety evaluation took place on Day 14. A Safety Review Committee reviewed safety, PK and PD data for each cohort of D-0120 dose level (2.5 mg, 5 mg, 10 mg, 20 mg) as well as when D-0120 5 mg was combined with 40 mg febuxostat. PK evaluation for multiple dose parameters included AUC_{0-τ}, C_{max}, C_{min}, T_{max} and FI.

Results: Dose escalation of D-0120 from 2.5 mg/day to 20 mg/day was completed without any dose limiting toxicities. Most AEs occurred during the study were mild to moderate in severity and did not require any treatment before resolution. There was no SAE and no dose reduction during the treatment period. The pharmacokinetic (PK) evaluation of ascending dose levels of D-0120 suggested a dose proportional increase in drug exposure and there was no significant change of PK profile between Day 1 and Day 7 of dosing. For pharmacodynamic (PD) evaluation, the serum uric acid (UA) levels before and after D-0120 dosing was evaluated on multiple days. The UA reduction effect achieved maximum at about 4-8 hours after dosing and the effect lasted for at least 24 hours. After the 7-day dosing period, the mean percentage of UA reduction from baseline showed an increasing trend as the dose level increased.

More detailed safety, PK and PD data from multiple D-0120 dose cohorts and D-0120/febuxostat combination cohort will be presented at the meeting.

Conclusion: The oral daily administration of a novel URAT1 inhibitor, D-0120, in healthy volunteers for 7 days was well tolerated at dose levels from 2.5 mg/day to 20 mg/day. The PK profile demonstrated a dose proportional increase. D-0120 administration for 7 days resulted in significant reduction of serum UA levels. Further evaluation of this novel agent in longer treatment period and in patients with hyperuricemia and/or gout is warranted.

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OP0206

ALLANTOIN - A BIOMARKER OF OXIDATIVE STRESS - IS HIGHER IN PATIENTS WITH GOUT THAN IN HEALTHY VOLUNTEERS, AND CORRESPONDS WITH SEVERITY OF DISEASE

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Background: Uric acid can be non-enzymatically oxidized into allantoin and other products by reactive oxygen species. Allantoin has emerged as a reliable biomarker for monitoring oxidative status both *in vitro* and *in vivo*¹. In gout patients, significantly increased plasma levels of allantoin have been found compared to healthy controls².

Objectives: The aim of this pilot study was to measure allantoin as a biomarker of oxidative stress in patients with gout using newly developed UHPLC-HILIC-MS/MS method³ and to investigate whether the allantoin levels are higher in patients with more severe disease (tophaceous gout).

Methods: We used clinical data and frozen serum (-80°C) from 10 patients with chronic tophaceous gout, 10 patients with chronic gout without tophi and 10 healthy controls. Allantoin was determined in serum with sensitive UHPLC-MS/MS method using an isotopically labeled internal standard as we described before³. In addition, the concentrations of serum CRP, creatinine and uric acid were measured. Data are summarized as medians with interquartile range [IQR]. Differences between two patient groups were evaluated using the Wilcoxon signed-rank test.

Results: The median concentrations of allantoin in the serum from patients with tophaceous gout were significantly higher than in patients with gout without tophi (4.2 [2.6] vs. 3.2 μM [1.5], $p = 0.0273$). There was no significant difference in other biochemical or demographic parameters (CRP, uric acid, creatinine, BMI, weight) between these two groups. Allantoin levels in healthy controls were significantly lower (0.5 vs. 4.2 [2.6], $p = 0.0020$, 0.5 vs. 3.2 [1.5], $p < 0.0001$) (Fig. 1).

Fig. 1. Box plots of allantoin concentration in patients with tophaceous gout with tophi, without tophi and in healthy controls.

Conclusion: We have observed significantly higher levels of serum allantoin in the patients with more advanced gout with tophi compared with the patients with chronic gout without tophi. We have found elevated values of allantoin in both groups in comparison with healthy controls. In our small cohort the level of allantoin correspond with the severity of disease presented by tophi. However, further studies in large cohorts are needed. We can speculate, whether higher level of oxidative stress may contribute to increased cardiovascular risk and mortality in patients with gout (and more so in severe gout)⁴.

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Cartilage - heal thyself!

OP0207

MECHANISM OF CHONDROPROTECTIVE EFFECTS OF 2-DEOXYGLUCOSE

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Background: We recently reported that the inhibitor of hyaluronan (HA) biosynthesis, 4-methylumbelliferone (4-MU) blocked IL-1β activation of MMP13 mRNA and protein expression in human osteoarthritic (OA), bovine as well as bovine or OA cartilage explants [1]. This was a somewhat counterintuitive observation

because we have also demonstrated that the overexpression of HAS2 (HAS2-OE) exerted the same chondroprotective effects on human and bovine chondrocytes. Others [2] have reported that HAS2-OE in tumor cells generates a flux in intracellular UDP-sugar pools that resulted in changes in cell metabolism; switching from a dependence on glycolysis to aerobic respiration. HAS2-OE and 4-MU likely also cause dramatic fluxes in intracellular UDP-GlcUA pools. From these results, we hypothesized that the effect of HAS2-OE and 4-MU relate to changing metabolism and the possibility of inhibition of glycolysis induce chondroprotective effect. To determine that, we used the glycolysis inhibitor, 2-Deoxyglucose (2DG) as an alternative agent to change metabolism in chondrocytes.

Objectives: The objective of this study was to investigate the mechanism of chondroprotective effects of 2DG

Methods: Bovine and human chondrocyte were stimulated with IL-1 β (2ng/ml) in the presence or absence of 4MU (1.0mM), 2DG (0.2-20mM). Bovine chondrocytes were tested using Seahorse Flux Analyzer (Agilent Tech) to determine rate changes in medium accumulation of +H protons (indicative of lactic acid accumulation: ECAR) and for O₂ consumption (indicative of mitochondrial respiration: OCR). Accumulation of MMP13 and phosphor AMPK (pAMPK) protein was quantified with Western blotting. Human and Bovine cartilage explants were cultured with L-1 β in the presence or absence of 2DG (20mM) and d 5-Aminimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR) to pharmacologically induce AMPK for 7 days and stained with Safranin O.

Results: Reduced mitochondrial potential and enhanced dependence on glycolysis was observed in IL-1 β stimulated chondrocytes. Co-treatment with 4-MU and 2DG returned the cell metabolism to levels at or below baseline (Fig 1A, B). The Seahorse ATP Rate Assay means the contributions of glycolysis and mitochondrial respiration to chondrocyte ATP production (Fig 1C). In control chondrocytes, the use of glycolysis contributes to the majority of ATP produced (grey bars) approximately 1/5th from the TCA cycle (red bars). IL-1 β -activated chondrocytes display increase in glycolysis and decrease in mitochondrial contributions. These changes are reversed by co-treatment with 4MU and 2DG. As shown in Figs 2A, 2DG reversed the IL1 β -induced increases accumulation of MMP13 protein in human OA chondrocytes by Western blotting analysis. Although IL-1 β lost safranin O staining in human and bovine samples, co-incubation with 2DG blocked in the loss of proteoglycan (Fig 2B). pAMPK is associate with energy homeostasis in chondrocytes. IL-1 β treatment decreased accumulation of phosphor AMPK. Co-treatment with 4-MU and 2DG resulted in a rescue of the pAMPK status (Figure 3A). Co treatment with AICAR, which is inducer of AMPK, also blocked in the loss of proteoglycan (Fig 3B).

FIGURE 1 Bovine chondrocytes were incubated 24h $\pm$ IL1 β (2 ng/ml) and without or with 4MU (1.0mM) or 2DG (2.0 mM). **Panel A** depicts a representative Mito Stress Test wherein bars represent basal and maximal mitochondrial respiration as a corrected OCR value. **Panel B** show summaries of ECAR data representative of changes in basal glycolysis rates as labeled. **Panel C** show a representative ATP rate assay wherein the contribution of glycolysis (grey bars) and mitochondrial respiration (red bars) to ATP production. (***: p<0.01 ****: p<0.001)

FIGURE 2 Effect of 2DG on chondrocytes activated with IL- β . **Panel A** represent the experiment with human OA chondrocytes treated $\pm$ 2 ng/ml IL1 β in the absence or presence of 0.2, 2.0 or 20 mM 2DG for 24h. **Panel B** depict representative examples of bovine and human OA cartilage explants stained by Safranin O.

FIGURE 3 The effect of 4MU and 2DG on p-AMPK changes in IL1 β activated chondrocytes.

Panel A: Bovine chondrocytes were treated for 24 h without (Ctr) or with IL1 β (2 ng/ml) and $\pm$ 2DG (2.0 mM), $\pm$ 4MU (0.5 mM).

Panel B depict representative examples of bovine cartilage explants stained by Safranin O treatment with AICAR or not.

Conclusion: 4-MU and 2DG have chondroprotective effect by changing metabolism and upregulate AMPK. We propose that 4MU and 2DG become useful when these endogenous responses are not enough to rescue cells from a pro-catabolic phenotype.

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Biological DMARDs in RA II

OP0208

PATIENTS REPORT FATIGUE AS AN ADVERSE DRUG REACTION OF BIOLOGICS

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Background: Chronic fatigue is a well-known symptom in patients with rheumatic diseases and other immune-mediated inflammatory diseases (IMIDs). Therefore, fatigue as an adverse drug reaction (ADR) to biologics may remain unrecognized or may erroneously be attributed to the disease.

Objectives: To assess patient-reported fatigue attributed to biologics for IMIDs and investigate predisposing factors of patient-reported fatigue.

Methods: The Dutch Biologic Monitor is a multicenter patient-reported ADR monitoring system that surveys patients using a biologic for an IMID. Patients completed web-based questionnaires regarding ADRs attributed to biologics and the course and experienced burden (5 point Likert scale) of these ADRs. All patient-reported ADRs with MedDRA Preferred Term 'fatigue' were defined as biologic-associated fatigue (BA-fatigue). Basic demographics and treatment characteristics were compared between patients reporting BA-fatigue and patients not reporting BA-fatigue (reported other ADRs or no ADRs).

Results: Out of 1369 participating IMID patients, 696 patients reported 1844 unique ADRs. BA-fatigue was reported by 100 patients and 48 patients described a consistent pattern of recurring fatigue after each administration. Most of these patients (88%) described recovery from BA-fatigue within one week after biologic administration and 73 patients reported health care