the medial femur (-64%), not at other surfaces and not in the total joint score (-16%).
Then, we treated collagenase-induced OA (CIOA) with paquinimod and evaluated the effects on day 42. In CIOA, synovial activation is high and S100A8/A9 levels in the synovium are significantly higher than those in DMM. Synovial activation was significantly reduced by paquinimod-treatment at the medial side of the patella-femur region (-57%). Osteophyte size was significantly reduced at the medial femur (-66%) and cruciate ligaments (-67%). Finally, OA-like cartilage pathology in CIOA was significantly reduced after paquinimod treatment on the medial side of both tibia and femur (-47% and -75% respectively) as well as in the total joint score (-46%) (Figure 1).
Conclusions: Paquinimod administered in the drinking water reduces synovial activation, osteophyte formation and OA-like cartilage pathology in CIOA. In contrast, in an experimental OA model where synovial activation is nearly absent (DMM), the effect of paquinimod is marginal. Paquinimod could prove a very promising treatment for osteoarthritis patients with high synovial activation by blocking S100A9.

882
A FUSION PROTEIN OF IL4 AND IL10 (IL4-10 SYNERKINE), IS VERY EFFECTIVE IN PROTECTING CARTILAGE FROM BLOOD-INDUCED DAMAGE

L.F. van Vulpen, M.E. van Meegeren, C. Steen-Louws, C.E. Hack, S.C. Mastbergen, F.P. Lafeber. *UMC Utrecht, Utrecht, Netherlands*

Purpose: Interleukin 4 (IL4), IL10 or the combination of both protects cartilage from blood-induced damage *in vitro*. Because IL4 and IL10 use different signaling pathways they are able to exert different, but also potentially additive effects. It has been postulated that they can act in synergy enhancing their beneficial effects and controlling their individual adverse effects. Therefore a combination of IL4 and IL10 might be the best option to administer *in vivo*.
To overcome the low bioavailability of IL4 and IL10 *in vivo*, a fusion protein has been developed: IL4-10 synerkine. It has been demonstrated that this novel drug inhibits numerous pro-inflammatory cytokines secreted by monocytes and T-cells. This study investigates whether this IL4-10 synerkine protects against blood-induced cartilage damage similarly as the combination of the individual components.
Methods: Human cartilage explants were exposed to 50% v/v whole blood for 4 days and simultaneously to a broad concentration range (0-100 ng/mL) of the IL4-10 synerkine. Effects of 10 ng/mL IL4-10 synerkine were compared to the same concentrations of the individual cytokines and the combination. Cartilage matrix proteoglycan turnover was assessed after a recovery period of 12 days. Moreover, the influence of IL4-10 synerkine (10 ng/mL) and its individual components on levels of IL1β and IL6 were investigated in a 4 days 50% v/v whole blood culture.
Results: Exposure to 50% v/v whole blood resulted in a clear statistical significant decrease of proteoglycan (PG) synthesis rate (-74%; $p = 0.012$) and an increase of PG release (+92%, $p = 0.017$) compared to control. Adding of IL4-10 synerkine, resulted in a clear dose-response curve, leading to full normalisation of PG synthesis rate and -release at higher concentrations (>10 ng/mL). These results were similar to the effect of the two individual cytokines combined (see figure). The individual cytokines had a more distinct profile where IL4 was superior over IL10 (see figure). Moreover, addition of IL4-10 synerkine reduced IL1β and IL6 production in whole blood cultures to control levels (both $p < 0.05$), similar to the combination of IL4 plus IL10 ($p = 0.180$ and $p = 0.173$, respectively).
Conclusions: IL4-10 synerkine strongly protects against blood-induced cartilage damage *in vitro,* presumably at least in part by reduction of pro-inflammatory cytokines including IL1β and IL6. Considering better bioavailability and more easy application of this synerkine compared to the individual cytokines, testing the IL4-10 synerkine in an *in vivo* model of blood-induced cartilage damage is warranted.

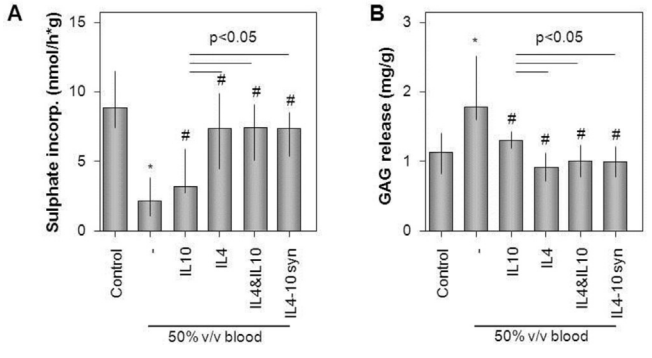

Figure – Effect of the IL4-10 synerkine compared to 1L4. IL10 and the combination of both cytokines (all 10ng/mL) on cartilage proteoglycan synthesis rate (A) and release (B) (n=8 individual donors). Median values ± interquartile range are depicted. Hash tags indicate a statistically significant difference compared to 50% v/v blood (p<0.05). asterisks indicate a statistically significant difference compared to control cartilage (p<0.05).

883
THE HIGHS AND LOWS OF TRANSLATIONAL DRUG DEVELOPMENT: ANTIBODY-MEDIATED INHIBITION OF ADAMTS-5 FOR OSTEOARTHRITIS DISEASE MODIFICATION

J. Larkin [†], T. Lohr [†], L. Elefante [†], J. Shearin [†], R. Matico [†], J-L. Su [†], Y. Xue [†], F. Liu [†], E.I. Rossman [†], J. Renninger [†], X. Wu [†], L. Abberley [†], R.E. Miller [‡], S. Foulcer [§], K.W. Chaudhary [†], C. Genell [†], D. Murphy [†], P.B. Tran [‡], S. Apte [§], A-M. Malfait [‡], C.C. Maier [†], C.J. Matheny [†]. [†] *GlaxoSmithKline, King of Prussia, PA, USA;* [‡] *Rush Univ., Chicago, IL, USA;* [§] *Cleveland Clinic Lerner Res. Inst., Cleveland, OH, USA*

Purpose: Aggrecanase activity, most notably ADAMTS-5, has been widely implicated as a pathogenic factor in cartilage degradation. Therefore, highly selective and potent monoclonal antibodies (mAbs) to both ADAMTS-5 and ADAMTS-4 were developed and experimental systems were utilized to assess target engagement and modulation of disease-related and safety endpoints with the intent of selecting a candidate and supporting clinical development in osteoarthritis (OA).
Methods/Results: In a surgical mouse model of OA, both ADAMTS-5 and ADAMTS-4-specific mAbs bound within cartilage following systemic administration, demonstrating access to the anticipated site of action, whereas structural disease modification and associated alleviation of pain-related behavior were only observed with ADAMTS-5 mAb treatment. Likewise, ex vivo treatment of human OA cartilage demonstrated a preferential role for ADAMTS-5 inhibition over ADAMTS-4, as measured by aggrecan-derived 374ARGS neoepitope release in explant cultures and was most evident in a distinct subset of patients where elevated 374ARGS neoepitope levels were present in explants prior to treatment. Notably, suppression of 374ARGS neoepitope release was sustained for weeks after a single treatment of human cartilage explants and in cynomolgus monkeys, consistent with a high affinity antibody/antigen interaction and slow ADAMTS-5 turnover that could translate to less frequent clinical dosing. As a result an ADAMTS-5 selective monoclonal antibody (GSK2394002) was progressed for preclinical development as a potential OA disease modifying therapeutic.
While desired pharmacology was observed in explant cartilage and disease models, ADAMTS-5 is also expressed in cardiovascular (CV) tissue. Recently it was reported that knockout mice show developmental heart valve defects and altered vascular and fibroblast proteoglycan processing. Along with these observations, an initial histologic cardiac signal in cynomolgus monkeys (subendocardial hemorrhage) prompted a careful assessment of CV function in a set of dedicated safety pharmacology studies. In these studies, increased mean arterial pressure (MAP) was observed within days after >3 mg/kg dosing and ST segment elevation was noted on 24 hour continuous ECG monitoring >30 mg/kg. Surprisingly, these effects were sustained for up to 8 months following a single dose and MAP was increased upon repeated low dose administration suggesting the effects are cumulative and, unlike the systemic pharmacodynamic marker signal, once induced they may not be readily reversible. Although a potential mechanistic link between ADAMTS-5 activity and versican processing is demonstrated, the cause of these cardiovascular observations is currently unknown. In a parallel general toxicology study, there were no histologic or biochemical correlates to the CV findings and no evidence of off-target mechanisms could be identified using in vitro tests examining cardiac conductance, ion channels, or binding selectivity in protein arrays.
Conclusions: The findings support a hypothesis set forth from knockout mouse studies that ADAMTS-5 is the major aggrecanase involved in