The SMOOS (SMOC2 in Osteoarthritis) project (number 302314) was run in the period of May 2012 to May 2014. This project aimed at deciphering the influence of the SPARC-related modular calcium binding protein 2 (SMOC2) in the pathophysiology of osteoarthritis. SMOC2 was originally identified by isolation from a chondrogenic extract of articular cartilage together, and Smoc2 is specifically expressed in the joint interzone. Proteomic analyses of osteoarthritic cartilage showed an increase in SMOC2.
Thus, our specific objectives were to study the possible role of SMOC2 in chondrogenesis and osteogenesis, as a recapitulation of endochondral ossification and subchondral bone alteration occur in osteoarthritis.
Moreover, we studied the role of SMOC2 in the onset and progression of osteoarthritis, using in vivo models refelecting this pathology.

Since the beginning of the project, we managed to fulfill the objectives set at the beginning of the projects.
Indeed, the in vitro part was performed in the chondrogenic ATDC5 mouse cell line and in the osteogenic MC3T3-E1 cell line. We managed to demonstrate that SMOC2 overexpression inhibited both chondrogenesis and osteogenesis, by affecting the Wnt and BMP signaling pathways. These data were confirmed in the by knocking-down SMOC2, which yielded an increase in the chondrogenesis phenomenon.
In the osteogenesis model, we further investigated the function of SMOC2 by creating mutant forms missing the calcium-binding domain or the follistatin-like domain, see figure 1.

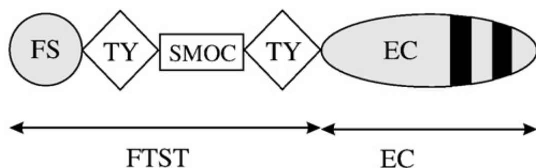

Figure 1. Domain structure of SMOC-2. The follistatin-like (FS) domain and the extracellular calcium-binding (EC) domain are separated by two thyroglobulin-like (TY) domains and the SMOC-specific domain. Reproduced from Maier et al., Exp Cell Res, 2008.

We managed to show that the overexpression of SMOC2 mainly mediates its inhibitory effects on calcification through the calcium-binding domain. We managed to transfer these overexpression datasets into primary human cells, i.e. using culture supernatant transfer onto periosteal-derived progenitors (figure 2) and endothelial cells.

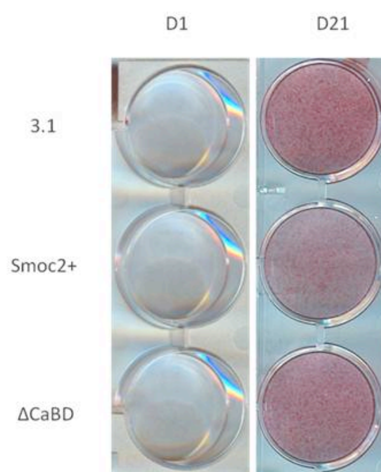

Figure 2. Alizarin red staining of differentiated human periosteal-derived cells. Culture supernatant from control MC3T3-E1 or cells overexpressing the full-length or calcium-binding mutant form was transferred onto human-periosteal derived cells during 21 days of osteogenic differentiation.

From these in vitro data on osteogenesis, we submitted a priority patent claim GB1308117.9 INHIBITOR OF CALCIFYING DISORDERS that was followed up by a full PCT "SPARC/smoc2 in calcifying disorders" (PCT/EP2014/059245) in collaboration with Leuven R&D, the KU Leuven technology transfer and legal office. We currently try to increase the attractivity for further licensing of this patent using an in vivo model of vascular calcification, that we will challenge with the full length and mutant forms of SMOC2.
The impact of this patent is potentially high, as vascular calcification is increasingly recognized as a clinically relevant problem, and is associated with aging, metabolic syndrome, type II diabetes and chronic kidney disease, all diseases with increasing prevalence. Over the last decade, research achievements have identified the complexity of vascular calcification and linked molecular processes involved with current concepts of bone and cartilage development. At the present time, targeted therapeutic or preventive interventions are lacking for cardiovascular-related calcification pathologies.
Concerning the in vivo part, we performed two models of osteoarthritis, namely the destabilization of the medial meniscus (DMM) and the papain-induced model. The DMM is seen presently as the gold standard reflecting the best the pathophysiology of osteoarthritis. A transection of the medial meniscus is performed, creating a mechanical instability of the knee joint of mice, which will develop osteoarthritic lesions within 12 weeks. The papain-induced model relies on the intra-articular injection of papain, an enzyme that will degrade the proteoglycan in the knee joint, creating a fast and severe instability. Osteoarthritic lesions are seen within seven days post-injection. After the indicated times above, mice are sacrificed and knees are collected, embedded and sectioned. This allows performing histological staining of proteoglycans and scoring of the osteoarthritic lesions (OARSI score).
These two osteoarthritis models were performed on wild type mice compared to heterozygous mice for SMOC2 (SMOC2+/-). Indeed, analysis of SMOC2-/- knockout mice was not possible as this gene invalidation is early embryonically lethal. The DMM model did not reveal any statistical difference between control and SMOC2+/- animals. However, the more severe papain-induced model showed that osteoarthritic lesions were increased in SMOC2+/- mice compared to controls. These data are in line with the in vitro chondrogenesis dataset, which demonstrated that a SMOC2 knockout increased chondrogenesis, thus yielding more hypertrophic chondrocytes. Follow-up research will correlate these results with immunohistochemistry analyses of specific markers within the cartilage of control and SMOC2+/- mice. In terms of impact, we plan to submit these data alongside the chondrogenesis, experiments in a research article to disseminate these findings.
Importantly, the in vitro experiments also provided a great platform and links to novel discoveries in interaction with teams that Dr. Cailotto had the opportunity to collaborate with. This resulted in two high-impact articles respectively demonstrating a critical role for histone methyltransferase DOT1L in cartilage homeostasis and genetics of OA (PNAS 2012) and for IGFBP3, an insulin-like growth factor binding protein (Annals of the Rheumatic Diseases 2014). These studies were perfectly aligned with the work on Smoc2 as interactions between these molecules and Smoc2 were studied and the same technology platforms were used.