culture. The authors assessed the role of PKC412 in the bone resorbing capacity of osteoclasts by culturing osteoclasts on dentine slices. To further characterise the effect of PKC412 on cell proliferation the authors performed MTT assays. The authors used quantitative PCR to evaluate expression levels of mRNA encoding for osteoclast specific markers such as nuclear factor of activated T cells cytoplasmic 1 (NFATc1), matrix metalloproteinase 9 (MMP9), Cathepsin K and TRAP. Flow cytometry analysis for annexin V and 7 Aminoactinomycin D (7-AAD) was performed to determine potential apoptotic effects of PKC412.

Results Increasing concentrations of PKC412 (IC50: 250 nM) dose-dependently reduced osteoclast numbers. Preosteoclasts were also significantly decreased after addition of PKC412, indicating an effect of PKC412 on early stages of osteoclastogenesis. In line with this finding, the authors showed a dose-dependent reduction of preosteoclast proliferation using MTT proliferation assays. Moreover, the authors detected a significant time- and dose-dependent increase in the ratio of apoptotic cells in the PKC412-treated cells by annexin V and 7-AAD staining. In the presence of PKC412 the authors obtained a significant reduction in osteoclast size, nuclei number and resorption pit formation. Consistently, the authors were able to demonstrate a dose dependent downregulation of mRNA coding for osteoclast markers, such as NFATc1, MMP9, Cathepsin K and TRAP in the presence of PKC412.

Conclusion These results suggest a regulatory role of PKC412 in preosteoclast differentiation und osteoclastogenesis through apoptosis induction.

A189 MULTI-TARGETED KINASE INHIBITOR PKC412 DIMINISHES OSTEOCLASTOGENESIS AND COUNTERACTS OSTEOCLAST FUNCTION IN VITRO

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Background Rheumatoid arthritis is a chronic inflammatory disease characterised by osteoclast mediated bone erosions. Small molecule multi-kinase inhibitors are being explored as potential therapeutic targets. PKC412 is a small molecule multi-kinase inhibitor targeting class III tyrosine-protein-kinases such as FMS-like tyrosine kinase 3 (FLT-3) and multiple isoforms of serine/threonine protein kinase C. PKC412 has been shown to inhibit macrophage function in vitro. However, the role of PKC412 in modulating the commitment of the monocyte/macrophage lineage to osteoclast precursors and their differentiation into mature osteoclasts has not been fully elucidated.

Objectives The authors aimed to investigate the effect of PKC412 on osteoclast differentiation and function.

Materials and methods The authors differentiated mouse bone marrow derived cells in the presence of macrophage colony-stimulating factor and RANKL into tartrate-resistant acid phosphatase positive (TRAP+) mononucleated preosteoclasts and TRAP+ multinucleated mature osteoclasts, and added PKC412 in increasing concentrations (10 nM to 10 μM) to the

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