STAT3 DEGRADERS INHIBIT TH17 DEVELOPMENT AND CYTOKINE PRODUCTION RESULTING IN PROFOUNDB HIBITION OF COLLAGEN-INDUCED AUTOIMMUNE MURINE ARTHRITIS

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Background: Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that belongs to a class of targets devoid of catalytic function, thus deemed “undruggable” by standard modalities such as small molecule inhibitors or biologics. STAT3 can be activated by various receptor- and non-receptor tyrosine kinases, playing a critical role in activation pathways triggered by cytokines, hormones, and growth factors, making it an attractive target for the treatment of inflammatory diseases.

Objective: Kymera has developed heterobifunctional molecules that selectively target STAT3 for degradation and elimination by the ubiquitin-proteasome pathway. We sought to evaluate the pharmacologic potential of these STAT3 degraders through in vitro and in vivo studies relevant to human autoimmune disease, including murine collagen-induced arthritis.

Methods: We evaluated the impact of STAT3 degraders on the activation of human monocytic, dermal fibroblasts, CD4+ T cells, and PBMCs by LPS, IL-6/IL-6R, IL-21, IL-23, as well as anti-CD3/CD28 plus a cocktail of cytokines and antibodies. STAT3 degradation and pSTAT3 inhibition were determined in comparison to a JAK1/2 small molecule inhibitor. Inhibition of cytokines, chemokines, and collagen release, as well as Th17 (CD4+/CD25−/γγ+CXCR6+) and Treg (CD4+/CD25+/CD127lowFOXP3+) expansion were used as anti-CD3/CD28 plus a cocktail of cytokines and antibodies. STAT3 degradation and pSTAT3 inhibition were determined in comparison to a JAK1/2 small molecule inhibitor. Inhibition of cytokines, chemokines, and collagen release, as well as Th17 (CD4+/CD25−/γγ+CXCR6+) and Treg (CD4+/CD25+/CD127lowFOXP3+) expansion were used as an in vitro efficacy assays. Finally, STAT3 degraders were tested in vivo, in a mechanistic (IL-6 chimeric) and a disease model (murine CIA) relevant to rheumatology indications.

Results: STAT3 degraders showed broad and potent activity in vitro against TLR receptor and cytokine-induced activation of immune and stromal cells, including soluble mediator release such as MCP-1/CCL2 and Collagen1α1. STAT3 degradation in CD4+ T cells robustly inhibited the development of Th17 cells, abrogating IL-17, IL-22, IL-8/CXCL8, and TNFα production, and increased Treg numbers as a percentage and total number. RANKL/OPG expression in FLSs of RA patients (RA-FLSs), thus reduces bone erosion in RA. Meanwhile, RANKL/OPG expression in FLSs of RA patients (RA-FLSs) was downregulated (Figure 1A). STAT6 phosphorylation and OPG upregulation of RA-FLSs by IL-13 can be significantly inhibited by a STAT6 inhibitor (inh.) (Figure 1B), IL-13 receptor α1 (IL-13Rα1) and IL-13 receptor α2 (IL-13Rα2) knockdown can inhibit OPG upregulation by IL-13 in RA-FLSs, indicating that IL-13 can induce OPG secretion in RA-FLSs through IL-13Rα1 and IL-13Rα2 via STAT6 pathway (Figure 1C). 20% conditioned medium of RA-FLSs pretreated by IL-13 significantly inhibited osteoclast differentiation in vitro. OPG knockdown in RA-FLSs significantly reversed the inhibition, indicating that IL-13 inhibits osteoclastogenesis by upregulating OPG in RA-FLSs (Figure 1D). In collagen-induced arthritis mice, IL-13 injection can reduce bone destruction in the ankle joint. Meanwhile, OPG expression in veminin positive FLSs was increased (Figure 1E and F).

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References:

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Background: Bone erosion in rheumatoid arthritis (RA) is partly caused by excessive activation of osteoclasts[1]. Osteoclasts can be derived from RA synovium and their differentiation can be inhibited by OPG, a decoy receptor of the osteoclastogenesis promoting cytokine RANKL[2]. Fibroblast-like synoviocytes (FLSs) are a main type of stromal cells in the synovium that can secrete OPG[4]. The OPG secretion by FLSs can be modulated by various cytokines[5]. Interleukin (IL)-13 is a cytokine rich in early RA synovial fluid and it decreases as RA progresses[6]. IL-13 was reported to alleviate bone erosion in RA mouse models[7]. However, how IL-13 reduces bone destruction remains unclear.

Objectives: To investigate if IL-13 can inhibit osteoclast differentiation by up-regulating OPG in FLSs of RA patients (RA-FLSs), thus reduces bone erosion in RA.

Methods: FLSs were isolated from the synovium tissue of RA patients with informed consent who underwent joint replacement surgery (Ethics No. 2021-544-01). OPG and RANKL expression by RA-FLSs were evaluated by qRT-PCR. OPG secretion was determined by ELISA. Western blot was performed to analyze OPG expression and the activation of STAT6 pathway. IL-13 and OPG siRNA pre-treated RA-FLSs conditioned medium were used in osteoclast induction to test if IL-13 can inhibit osteoclastogenesis by up-regulating OPG in RA-FLSs. Micro-CT scanning and immunofluorescence were done to reveal if IL-13 can induce OPG expression and alleviate bone erosion in vivo.

Results: IL-13 can significantly upregulate OPG expression and secretion by RA-FLSs. Meanwhile, RANKL/OPG expression in FLSs was downregulated (Figure 1A). STAT6 phosphorylation and OPG upregulation of RA-FLSs by IL-13 can be significantly inhibited by a STAT6 inhibitor (inh.) (Figure 1B), IL-13 receptor α1 (IL-13Rα1) and IL-13 receptor α2 (IL-13Rα2) knockdown can inhibit OPG upregulation by IL-13 in RA-FLSs, indicating that IL-13 can induce OPG secretion in RA-FLSs through IL-13Rα1 and IL-13Rα2 via STAT6 pathway (Figure 1C). 20% conditioned medium of RA-FLSs pretreated by IL-13 can significantly inhibited osteoclast differentiation in vitro. OPG knockdown in RA-FLSs significantly reversed the inhibition, indicating that IL-13 inhibits osteoclastogenesis by upregulating OPG in RA-FLSs (Figure 1D). In collagen-induced arthritis mice, IL-13 injection can reduce bone destruction in the ankle joint. Meanwhile, OPG expression in veminin positive FLSs was increased (Figure 1E and F).

Conclusion: IL-13 can inhibit osteoclast differentiation by up-regulating OPG in RA-FLSs through IL-13 receptors via STAT6 pathway, thus ameliorate bone erosion in RA.