Inhibition of the Epigenetic Reader BRD4 Reduces Extracellular Matrix Deposition in an In-Vitro Model for Knee Arthrofibrosis

Mason F. Carstens, M.S., Oliver B. Dilger, B.A., Cole E. Bothun, B.S., Emily A. Schenk., Benjamin D. Mallinger, B.S., Isabella G. Helgeson, B.S., Hassan Ahad, B.S., Adam E. Pagnano, Mark E. Morey, M.D., Joaquin Sanchez-Sotelo, M.D., Ph.D., Daniel J. Berry, M.D., Roman Thaler, Ph.D., M.S., Matthew P. Abdel, M.D.
Mayo Clinic, Rochester, MN
carstens.mason@mayo.edu

Disclosures: MFC (N) OBD (N), CEB (N), EAS (N), BDM (N), IGH (N), HA (N), AEP (N), MEM (Bonebridge, Zimmer Biomet, Global Elbow Network), JSS (Parvizi, Precision OS, ACUMED, JSES, ASES, Stryker, Exactech, Orthobullets, JBJS, Elsevier), DJB (DePuy, Elsevier, Wolters Kluwer, J&J, OREF), RT (N), MPA (IOEN, AAHKS, Hip Society, Stryker)

INTRODUCTION: Arthrofibrosis is a complication of total knee arthroplasty (TKA) that is characterized by reduced knee range of motion due to excessive scar tissue deposition in the joint. Although the cytokine TGF-β1 is known to initiate profibrotic pathways, the epigenetic reader BRD4 has also been connected to fibrotic processes. BRD4 contains a bromodomain extra terminal domain (BET) that reads acetylated lysine residues on histone proteins. Since BRD4 has been implicated in fibrosis before, it may be a potential therapeutic target for arthrofibrosis. A promising competitive inhibitor of BRD4 is CPI-203, which specifically targets the BET domain of the protein. It is hypothesized that the inhibition of the BET domain of BRD4 prevents TGF-β1 induced myofibroblastogenesis, which entails increased extracellular matrix (ECM) deposition and cytoskeletal dysregulation. As such, CPI-203 may be effective in preventing arthrofibrosis in the context of TKA.

METHODS: Following Institutional Review Board (IRB) approval, 8 patients (4 female and 4 male) undergoing primary TKA and 8 patients (4 female and 4 male) undergoing revision TKA for arthrofibrosis were prospectively selected for tissue collection. For the primary TKA patients, the suprapatellar pouch was collected and for the arthrofibrosis patients, scar tissue was excised and collected as well. All tissues were digested in a 2 mg/mL collagenase I digestion and the adhering fibroblasts were cultured in Advanced MEM medium supplemented with 5% human platelet lysate. Passage 2 and 3 cells were utilized in this study. To determine an effective dose for reducing ECM with CPI-203 treatment, a serial dilution that ranged from 300 nM to 37.5 nM was conducted in the presence (**Fig. 1A-F**) and absence (**Fig. 2A-F**) of 10 ng/mL TGF-β1, with vehicle solutions (DMSO for CPI-203 and HCl for TGF-β1) acting as control. MTS assay (metabolic activity) and Hoechst stain (DNA as proxy for cell number) were utilized as cytotoxicity readouts and Picrosirius Red staining was used to measure ECM deposition after removal of cell bodies with 0.05% w/v deoxycholate. All measurements were conducted after 3 days of CPI-203 treatment. A 150 nM CPI-203 dose was chosen as the effective experimental dose for further experiments. Metabolic activity and collagen deposition per cell were calculated using Hoechst as a proxy for cell number to account for potential differences in cell number across wells. Experimental groups were normalized to vehicle
(control) and one-way ANOVA with Tukey's multiple comparisons, or t-test was used to detect significant differences between the groups.

RESULTS SECTION: In arthrofibrosis patients derived cells, TGF-β1 alone increased cell number by 5.9%, and the addition of 150 nM CPI-203 decreased cell number by 3.9% when compared to vehicle, as measured by Hoechst staining (**Fig. 3A**). The metabolic activity/cell was increased by 10% (p= 0.01) for TGF-β1 and by 25.2% (p= 0.0002) for CPI-203 + TGF-β1 treatment compared to vehicle (**Fig. 3B**). CPI-203 reduced the TGF-β1 mediated collagen deposition/cell by 79% (p= <0.0001) (**Fig. 3C**). In the absence of TGF-β1, total Hoechst showed a 6.7% (p= <0.0001) decrease in cell number (**Fig. 3D**), a 4% (p= 0.04) increase in metabolic activity/cell (**Fig. 3E**), and a 21.5% (p= <0.0001) decrease in collagen deposition/cell compared to vehicle (**Fig. 3F**). For primary TKA patients, the total Hoechst showed a 3.3% (p= 0.06) increase in cell number for TGF-β1 and a 4.4% (p= 0.06) decrease in cell number for the CPI-203 + TGF-β1 treatment compared to vehicle. The metabolic activity/cell was increased by 21.1% (p= 0.004) for TGF-β1 and 27.2% increased (p= 0.0008) for CPI-203 + TGF-β1 treatment compared to vehicle. CPI-203 + TGF-β1 reduced the TGF-β1 effect by 84.8% (p= 0.0005) for collagen deposition/cell. In the absence of TGF-β1, total Hoechst showed a 5.5% (p= 0.003) decrease in cell number, a 14.2% (p= 0.003) decrease in metabolic activity/cell, and a 13.4% (p= 0.005) decrease in collagen deposition/cell compared to vehicle.

DISCUSSION: This study demonstrates that the BRD4 inhibitor CPI-203 significantly reduces TGF-β1-induced collagen deposition in human knee fibroblasts by at least 80%. CPI-203 also exhibited reduction in ECM independent of TGF-β1. These findings support CPI-203 as a promising candidate for mitigating arthrofibrosis though further *in vitro* validation is needed.

SIGNIFIGANCE/CLINICAL RELEVANCE: Our results suggest CPI-203 could be a novel therapeutic agent that by targeting BRD4 prevents pathological scar tissue formation. This could improve knee range of motion, promote better functional recovery, and reduced need for revision surgeries following TKA for patients at risk of arthrofibrosis.

IMAGES AND TABLES:

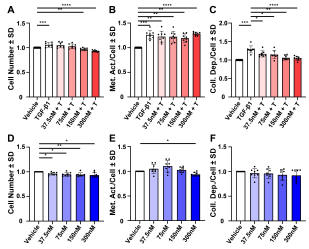

Figure 1. Dose response curves for arthrofibrosis cells. **(A, D)** Hoechst, **(B, E)** metabolic activity/cell, **(C, F)** collagen deposition/cell in the presence and absence of TGF-β1. T = TGF-β1.

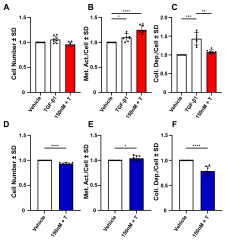

Figure 3. Arthrofibrosis cells treated with 150nM CPI-203. **(A, D)** Hoechst, **(B, E)** metabolic activity/ cell, **(C, F)** collagen deposition/cell in the presence and absence of TGF-β1. T = TGF-β1.

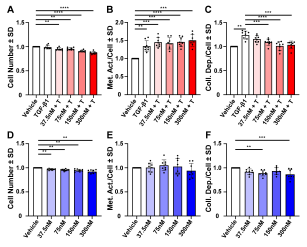

Figure 2. Dose response curves for primary TKA cells. **(A, D)** Hoechst, **(B, E)** metabolic activity/cell, **(C, F)** collagen deposition/cell in the presence and absence of TGF-β1. T = TGF-β1.

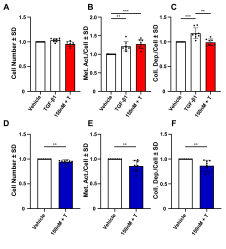

Figure 4. Primary TKA cells treated with 150nM CPI-203. **(A, D)** Hoechst, **(B, E)** metabolic activity/ cell, **(C, F)** collagen deposition/cell in the presence and absence of TGF-β1. T = TGF-β1.