Background: The pathophysiology of fibrosis, a hallmark of diseases like Systemic Sclerosis, is still not well understood. The role of epigenetic modifications in the pathogenesis of fibrosis is increasingly explored. DOT1L, the unique H3K79-methyltransferase, regulates histone modification at the Lysine residue at position 79, thereby controlling gene expression programs. Inhibition of DOT1L in cartilage and bone has cell-type specific effects on Wnt signaling, a pathway suggested to play an important role in fibrotic disease.

Objectives: To study the changes in the transcriptome of dermal fibroblasts after DOT1L inhibition.

Methods: Primary human dermal fibroblasts isolated from abdominal skin were treated with DOT1L-inhibitor EPZ-5676 or vehicle and stimulated or not with TGF-beta or CHIR99021 (activating Wnt signaling). RNAseq was performed using the NextSeq500 as a platform and TruSeq as library prep kit. The preprocessed reads were aligned to the reference genome using the Nucleomics Core (www.nucleomics.be).

Results: The DOT1L-inhibitor EPZ-5676 effectively reduced H3K79 dimethylation in the treated samples. RNAseq analysis revealed 663 differentially expressed genes (DEG) was done using the edgeR package in R version 3.20.9. The resulting p-values were corrected for multiple testing with Benjamini-Hochberg to control for false discovery rate (FDR). DEG were selected based on FDR-value less than 0.05 and a fold change of an absolute log2-ratio larger than 1. Pathway analysis was done using PANTHER14.0. Western Blot for di-methylated H3K79 was performed to confirm successful DOT1L inhibition.

Conclusion: More than 600 differentially expressed genes were discovered by RNA sequencing in human dermal fibroblasts exposed to DOT1L inhibition (independent of TGF-beta or CHIR 99021 stimulation). These gene lists and their networks indicate that DOT1L activity affects integrin signaling, the cadherin and Wnt pathway, suggesting a potential impact on the fibrotic process that is leading to pathology in SSC.