Response to: 'Correspondence on 'Machine learning integration of scleroderma histology and gene expression identifies fibroblast polarisation as a hallmark of clinical severity and improvement" by Manetti

We thank Dr Manetti for his comments regarding the distinctive CD34 positive dermal stromal cell population and their pathological changes in systemic sclerosis (SSc) skin.[1–4] One challenge for the field of SSc dermatopathology is a lack of broad consensus regarding the terminology used to describe CD34+ cells.[5] Indeed, CD34+ cells have been described as fibrocytes,[6] stromal fibroblastic cells,[7] dermal dendritic cells,[8] fibroblasts[5 9–11] and telocytes.[3 4] Our work adds to prior observations by noting that skin samples that exhibit a loss of CD34+ cells, gain expression of alpha-smooth muscle actin (aSMA) in a similar microanatomic distribution, specifically the deeper dermis and subcutaneous fat. This pattern of CD34 and aSMA reversed as patients improved clinically and was associated with a gene signature relevant to clinical improvement.[12] Our study was not able to conclude whether this polarisation of aSMA and CD34 staining in scleroderma skin represents aSMA+ cells that transdifferentiated into CD34+ cells over time or aSMA+ cells that died and were replaced by new CD34+ cells. Manetti *et al* conducted a comprehensive immunophenotyping and structural study of CD34+ cells in SSc and did not observe costaining of CD34 with CD90 or aSMA.[3] However, other studies of SSc, scarring, and tissue repair have identified CD90+/CD34+ cells,[7 9] CD90+/aSMA+ cells,[7 9] and CD34+/aSMA+ cells,[10] supportive of the fibroblast transition hypothesis. When examining biopsies with the various aSMA and CD34 staining patterns, we recognise that aSMA+ cells differ morphologically from CD34+ cells. Specifically, aSMA+ cells are larger than the CD34+ cells, exhibiting a rather abundant eosinophilic cytoplasm and vesicular chromatin.

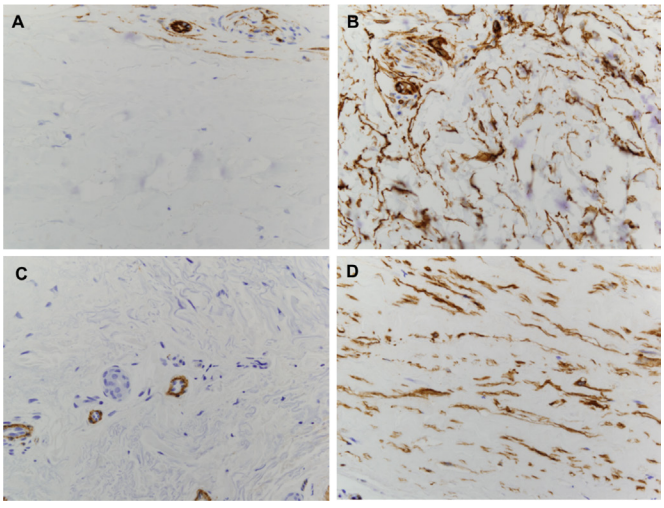

Figure 1 Alpha-smooth muscle actin (aSMA) and CD34 staining patterns of four diffuse cutaneous systemic sclerosis skin biopsies. (A) Biopsy with low CD34 staining where endothelial cells were excluded from overall CD34 score (magnification 40×). (B) Biopsy with high CD34 staining demonstrating elongated cytoplasmic processes (magnification 40×). (C) Biopsy with low aSMA staining (magnification 40×). (D) Biopsy with high aSMA staining demonstrating distinct morphological features compared with B, including larger cell size and abundant cytoplasm (magnification 40×).

As Dr Manetti highlights, a study limitation includes that we did not perform concurrent CD31 staining which may have resulted in the incorporation of some CD34+ endothelial cells into the CD34 staining score. However, the interstitial intervascular CD34 staining pattern contrasts the well-defined microvascular staining, highlighted by CD34 and CD31. In severe scleroderma, the negative CD34 staining pattern in the intervascular interstitium is in contradistinction to the strongly positively staining vessels, the latter serving as an important positive internal control (figure 1).

In summary, it is increasingly clear that multiple, distinct stromal cell and fibroblast populations play a role in SSc skin, and at least nine types of dermal fibroblasts have been identified by single-cell RNA sequencing in various skin diseases.[13] This underscores that a more detailed understanding cellular heterogeneity may be essential for understanding SSc disease pathogenesis and clinical phenotypes. We look forward to future studies using higher dimensional analyses such as single-cell RNA sequencing and multiparameter immunofluorescence staining to further characterise SSc cell types and international collaborative efforts to come to consensus regarding nomenclature.

Kimberly Showalter ,[1] **Cynthia Magro,**[2] **Yaxia Zhang,**[3] **Robert Spiera,**[1] **Dana E Orange** ,[1,4] **Jessica K Gordon**[1]

[1]Department of Medicine, Division of Rheumatology, Hospital for Special Surgery, New York, New York, USA
[2]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, New York, USA
[3]Department of Pathology and Clinical Laboratory Medicine, Hospital for Special Surgery, New York, New York, USA
[4]Center for Clinical and Translational Science, Laboratory of Molecular Neuro-Oncology, The Rockefeller University, New York, New York, USA

Correspondence to Dr Kimberly Showalter, Department of Medicine, Division of Rheumatology, Hospital for Special Surgery, New York, NY 10021, USA; showalterk@hss.edu

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Twitter Dana E Orange @danaorange

Contributors KS and YZ wrote the first version of the manuscript. All authors read, critically revised and approved the final manuscript. DEO and JKG contributed equally.

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ORCID iDs
Kimberly Showalter http://orcid.org/0000-0001-9343-5545
Dana E Orange http://orcid.org/0000-0001-9859-7199

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