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Letter to the Editor

Investigation of morphological, vascular and biochemical changes in the skin of an atopic dermatitis (AD) patient in response to dupilumab using raster scanning optoacoustic mesoscopy (RSOM) and handheld confocal Raman spectroscopy (CRS)

1. Case report

Dupilumab is a fully human monoclonal IgG4 antibody that inhibits the IL-4 and IL-13 signal pathways [1]. It was approved on March 28, 2017 by US Food and Drug Administration (FDA) for treatment of moderate to severe atopic dermatitis (AD). It has a good treatment response with improvements in both physician reported outcome measures such as Eczema Area Severity Index (EASI) and patient reported outcomes measures such as Dermatology Life Quality Index (DLQI) and pruritus scores [2]. Maximal clinical improvement in AD patients treated with dupilumab was seen at about 4–6 weeks [3], and improvements in molecular signature of lesional skin was seen at 4 weeks [4].

Non-invasive skin bio-imaging modality techniques such as raster-scanning optoacoustic mesoscopy (RSOM) and confocal Raman spectroscopy (CRS) allows for the examination of skin on an ultrastructural level. It affords the opportunity to appreciate three dimensional anatomical, vascular and molecular changes of the skin in vivo over time, giving valuable information for understanding the mechanism of action of treatments such as dupilumab. RSOM, which is based on optoacoustic imaging, combines the advantages of deep tissue interrogation of ultrasound and high optical contrast [5]. It provides objective characterization of the severity of AD by visualizing skin morphology and vascular patterns in the epidermis and dermis, enabling quantification of inflammation in a label free manner [6]. Conversely, CRS measures vibrational modes of molecules, and is used to assess the molecular composition of the skin in AD patients [7]. We have developed a handheld CRS system for skin characterization, which allows detection of constituents at different skin layers with high accuracy. These two optical techniques can be used together in a complementary fashion to detect anatomical, vascular and biochemical changes in skin, to characterize inflammatory skin diseases like AD.

Herein, we report the treatment response and changes in skin using RSOM imaging and CRS in a 67-year-old Chinese male with moderate AD treated with dupilumab. He was diagnosed with AD since age six and had previously used topical corticosteroids therapy and phototherapy with minimal response. At baseline, his body surface area of involvement was 15% and EASI and DLQI scores were 12.9 and 21 respectively. He was started on dupilumab at the approved dosing of 600 mg as loading dose followed by 300 mg every 2 weeks. Baseline and week 4 RSOM imaging and raw CRS measurements were performed over specified lesional area located at the intersection of the mid-point from his right volar wrist and right cubital fossa skin crease, and the midpoint of the width of the forearm (Fig. 1a). Measurements were conducted at constant temperature and humidity settings. The acquired raw Raman spectra were pre-processed for noise removal, before being fed into an in house developed non-negative least squares spectral un-mixing algorithm. The raw composite Raman spectra were fitted to a linear combination of the contributions from the individual skin constituents, such as ceramide, urocanic acid, water, and various amino acids as part of natural moisturizing factors. At Week 4, his body surface area of involvement had decreased to 10% and his EASI and DLQI scores improved to 3.30 and 13 respectively. While the forearm skin appeared grossly similar after dupilumab treatment (Fig. 1a), an approximate increase of 1077% (tenfold) in ceramide, 52% in water and 73% in urocanic acid content were seen using CRS, reflecting the restoration of the skin barrier function (Fig. 1b–d). RSOM images showed an irregular and thickened epidermis prior to treatment compared to a regular and less thickened epidermis post-treatment (Fig. 2a–b). Compared to baseline, post-treatment images also showed a reduction in the number of enlarged capillary loops, decrease in total blood volume (TBV) and diameter of dilated vessels, owing to subsided inflammation. Quantitatively, we observed a reduction of 32% in epidermis thickness (Fig. 2c), 10% in TBV (Fig. 2d) and 26% in vessel diameter in the dermis (Fig. 2e). Post treatment, there was also a reduction in trans-epidermal water loss (TEWL) from 22.6 to 13.8 g/m [2]/h, decrease in total IgE concentration from 5447 IU/mL to 2433 IU/mL, and absolute eosinophil count from 270 to 180 cells/μL. In short, we demonstrated the complementary application of RSOM imaging and CRS to characterize the positive response to dupilumab treatment in AD skin, which correlated well with improvement in TEWL, IgE and eosinophil count.

The innovative nature of the RSOM technology lies in its capability for volumetric, quantitative differentiation of skin layers and vasculature. It enables tomographic three-dimensional imaging of skin up to ˜2 mm depth with a spatial resolution of ˜ 30 microns, providing tremendously high optical contrast, owing to the strong absorption of blood and melanin at 532 nm laser

Abbreviations: AD, atopic dermatitis; RSOM, raster scanning optoacoustic mesoscopy; CRS, confocal Raman spectroscopy; EASI, Eczema Area Severity Index; DLQI, Dermatology Life Quality Index and TBV, total blood volume.

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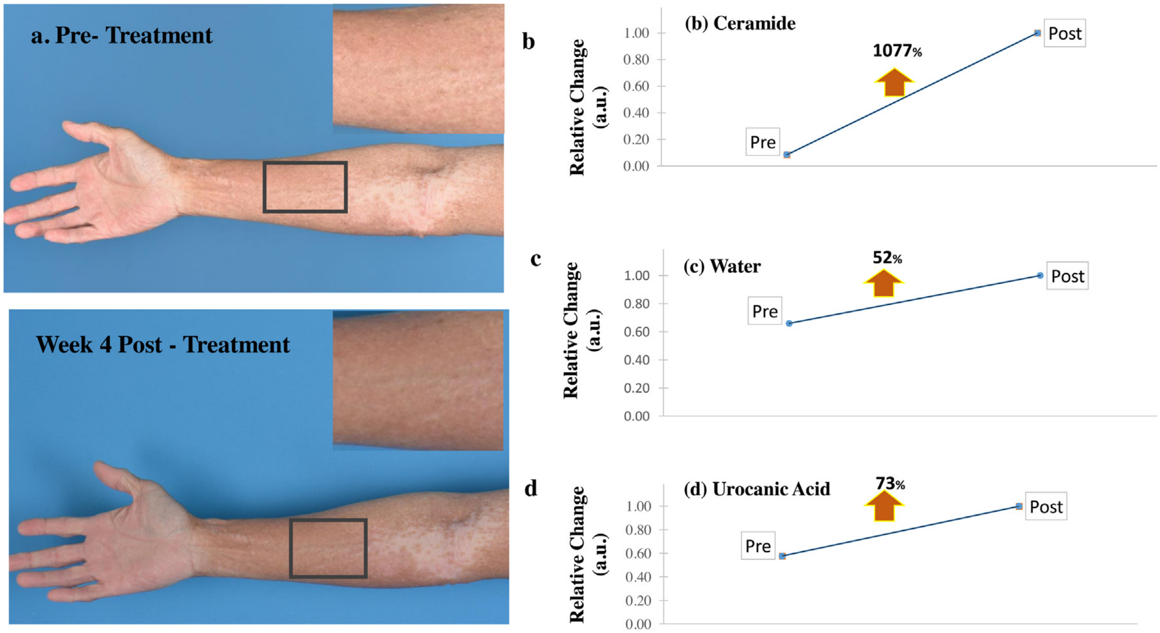

Fig. 1. **(a)** Clinical images of the patient's right forearm before and at week 4 of dupilumab treatment. Intersections of the arrows reflect the prespecified area for RSOM and CRS measurement. Magnified insets show a grossly similar clinical appearance before and after treatment. **(b–d)** Objective increases in ceramide, water and urocanic acid levels measured in skin using CRS after dupilumab treatment.

excitation [6]. Imaging depth and spatial resolution of RSOM is superior to modern forms of optical microscopy and hence it is emerging as an ideal imaging technique for spatial mapping of skin vasculature. CRS also offers competitive advantages over existing molecular characterization methods such as skin biopsy, in terms of being non-invasive, label-free and quick.

This report illustrates the contributions of non-invasive skin characterization techniques in our understanding of AD. These optical techniques can detect subclinical skin changes that may not be apparent clinically, and allows repeated measurements over time. Hence, they couldpotentially serve as treatment monitoring targets in addition to serum biomarkers [8]. Its application can also

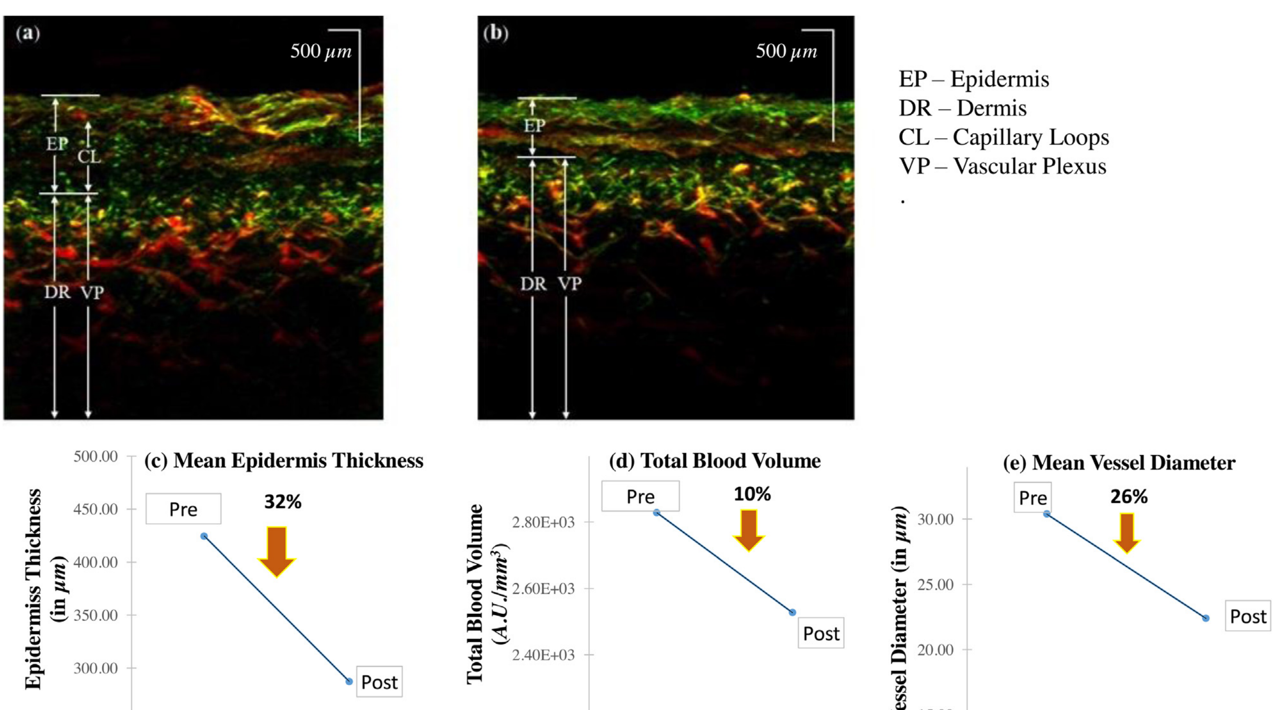

Fig. 2. RSOM images and quantification of skin characteristics. RSOM images of the skin at baseline **(a)** and 4 weeks after dupilumab treatment **(b)** showing a decrease in epidermis thickness, decrease in dilated capillary loops and diameter of vessels, and increased epidermis regularity. The cutaneous blood vascular architecture consists of a lower and an upper horizontal plexus. The capillary loops (CL) extends from the former and can be seen from the 'dot'-like structures in the image while the bigger vascular plexus (VP) forms the lower horizontal plexus. Red and green represents the larger and smaller vascular structures respectively. All scale bars: 500 μm. **(c–e)** Objective reduction in epidermis thickness, total blood volume and mean vessel diameter after dupilumab treatment.

be extended to the imaging of other inflammatory skin diseases [9] and changes in response to emerging targeted therapies in the horizon [10]. With a larger sample size and prospective study design, we might also be able to identify certain skin prognostic features of the different biological therapies targeting various signal transduction pathways.

As we move into an era of targeted therapies for the treatment of chronic inflammatory skin diseases, the availability of non-invasive skin characterization techniques offers great potential in better understanding of the disease and its response to treatment.

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Declaration of Competing Interest

Professor Vasilis Ntziachristos has equity ownership and is a member of the advisory board of iThera medical GmbH. Other authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.07.003.

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