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Reflectance confocal microscopy in the diagnosis of pigmented macules of the face: differential diagnosis and margin definition

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In vivo reflectance confocal microscopy (RCM) is a noninvasive high-resolution skin imaging tool that has become an important adjunct to clinical exam, dermoscopy and histopathology assessment, in the diagnosis and management of pigmented macules of the face. The diagnosis of early stage lentigo maligna (LM) and lentigo maligna melanoma (LMM) is challenging and RCM improves the diagnostic accuracy in the differential diagnosis of LM with other macules of the face such as solar lentigo (SL), pigmented actinic keratosis (PAK), seborrheic keratosis (SK) and lichen planus-like keratosis (LPLK). Here we review the state-of-the-art of RCM morphologic descriptors, standardized terminology, and diagnostic algorithms for the RCM assessment of pigmented macules of the face including melanocytic, and nonmelanocytic lesions. Clinical applications of RCM are broad and include diagnosis, assessment of large lesions on cosmetically sensitive areas, directing areas to biopsy, delineating margins prior to surgery, detecting response to treatment and assessing recurrence. The present review is intended to summarize the application of RCM for the correct diagnosis of challenging pigmented facial macules and to evaluate its application in LM margin mapping during the pre surgical phase.

Introduction

Differential diagnosis of lentigo maligna (LM) and lentigo maligna melanoma (LMM) from non melanocytic skin neoplasm (NMSN) presenting as pigmented macules of the face, including solar lentigo (SL), pigmented actinic keratosis (PAK), seborrheic keratosis (SK) and lichen planus-like keratosis (LPLK), is of utmost importance. First, diagnostic ambiguities of LM diagnosed as an NMSN can lead to inappropriate management and delayed melanoma diagnosis.[1] Further, erroneous diagnosis can lead to unnecessary excisions of LM-like benign macules, resulting in surgical morbidity, especially in elderly patients, and avoidable facial cosmetic issues.

Dermoscopy has been proven to improve diagnostic accuracy compared with the unaided eye.[1] Dermoscopy has therefore become an integral part in the clinical examination of pigmented facial macules.[2] However, in some cases, differential diagnosis of facial LM from NMSN still remains challenging.[3]

Reflectance confocal microscopy (RCM) represents an innovative, non-invasive tool for dermatological imaging. This technique enables *in vivo* observation at a *quasi*-histological resolution of the epidermis, dermo-epidermal junction (DEJ) and upper dermis, providing horizontal grayscale color images, related to refractive index of different tissues and cell structures. The highest refractivity is shown by melanin, contained in melanosomes, melanocytes, melanophages and pigmented keratinocytes, followed by structures containing keratin. Of note, tissue cellular contrast can also be visualized when melanin is present in very small quantities, thanks to the brightness of some subcellular organelles or surrounding structures.[4,5]

RCM can assist in differential diagnosis of pigmented facial macules, especially in the presence of ambiguous dermoscopy criteria,[6,7] particularly when the differential diagnosis between LM/LMM and NMSK (with well known dermoscopy criteria) is hindered.[3,8] Additionally, RCM has been shown to assist in LM margin mapping during pre-surgical work-up.[9–11]

The present review is intended to summarize the application of RCM for the correct diagnosis of pigmented facial macules and to evaluate its application in LM margin mapping during the pre surgical phase.

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RCM diagnosis of pigmented facial macules

Actinic keratosis

Pigmented actinic keratosis (PAK) is a variant of actinic keratosis (AK) characterized by various degrees of brown-gray pigmentation and a smooth or slightly scaly surface, which spreads radially. PAK diagnosis can be challenging, as it often mimics other pigmented lesions such as SL, SK, LPLK and LM.[12] At dermoscopy, the accumulation of the intra-epidermal pigment in pigmented lesions can inhibit the detection of subsurface structures, whereas RCM can visualize the underlying architecture and cytology of the skin also in the presence of hyperpigmented areas.[12,13] PAK are characterized by areas of an atypical honeycomb pattern at the granular and spinous cell layer, irregularly shaped keratinocytes, mainly located outside the infundibulum, which is constituted of apparently regular and monomorphous cells. Further, numerous dendritic cells are present in the epidermis, in the area between hair follicles, without infiltrating the infundibulum. Furthermore, Nascimento *et al.* demonstrated that the brown pseudonetwork surrounding the inner grey halo (IGH) associated with PAK at dermoscopy evaluation, showed numerous melanocytes in the inter-follicular epidermis, corresponding to bright dendritic cells, and pigmented keratinocytes in the basal layer of hair follicles at RCM evaluation.[14] Thus, the pigmentation of the basilar keratinocytes surrounding the hair follicle seems to be responsible for the appearance of the IGH (Tyndall effect)[14] (Fig. 1).

Moreover, RCM permits non-invasive stratification of patients in relation to microscopic lesion characteristics and accurate follow up during therapy, permitting the demonstration of the clearance of clinical and subclinical lesions at the different stages of treatment.[15,16]

Solar lentigo, lichen planus-like keratosis, seborrheic keratosis

Benign non melanocytic lesions, including LPLK, SL, and SK, usually present as large pigmented macules mostly occurring on the face and sun-exposed areas.[6,17,18] The clinical distinction from malignant lesions, such as LM/LMM, PAK and pigmented basal cell carcinoma (BCC) can be challenging because they share many clinical and dermoscopic features.[6]

RCM criteria for classic SL and SK were defined in 2012.[19] The main RCM features observed in the SK include a regular honeycomb pattern at the suprabasal levels of the epidermis indicating absence of significant atypia of keratinocytes, epidermal projections and keratin-filled invaginations of the lesion surface attesting to the papillated surface of SK, corneal pseudocysts at the suprabasal levels of the epidermis, round to polymorphous densely packed and well-circumscribed dermal papillae at the DEJ indicating an undulating DEJ architecture, cords with bulbous projections at the DEJ indicating elongated, bulbous rete ridges, and a mixed vascular pattern with dilated round and linear blood vessels and melanophages at the papillary dermis.[19–21] Longo *et al.* described a series of SK which showed an overall RCM architecture represented by a clod pattern (matching with the clustered globules seen in dermoscopy), as is commonly seen in melanocytic nevi.[12,22,23] However, at closer inspection, the clods appeared as compact nests of keratinocytes rather than melanocytes. Keratinocytes are usually polygonal in size whereas melanocytes are larger and with variable morphology (roundish or dendritic shaped cells). However, a clear-cut distinction between keratinocytes and melanocytes is not always easy and reliable.[22]

Bassoli *et al.* demonstrated that LPLK are characterized by typical honeycomb pattern, elongated cords at the DEJ and numerous bright stellate spots or plump-bright cells in the superficial dermis.[24]

Taking into account the RCM features of skin neoplasms in differential diagnosis, the diagnosis of LPLK can be done when a solitary lesion shows all of the following criteria: typical honeycomb pattern, elongated cords at the DEJ and numerous bright stellate spots/plump-bright cells in the superficial dermis, and an absence of melanocytic or other NMSC features, as such as bright nucleated or dendritic cells in the epidermis, bright dendritic, spindle or atypical cells at the DEJ, and tumour islands in the superficial dermis.[24]

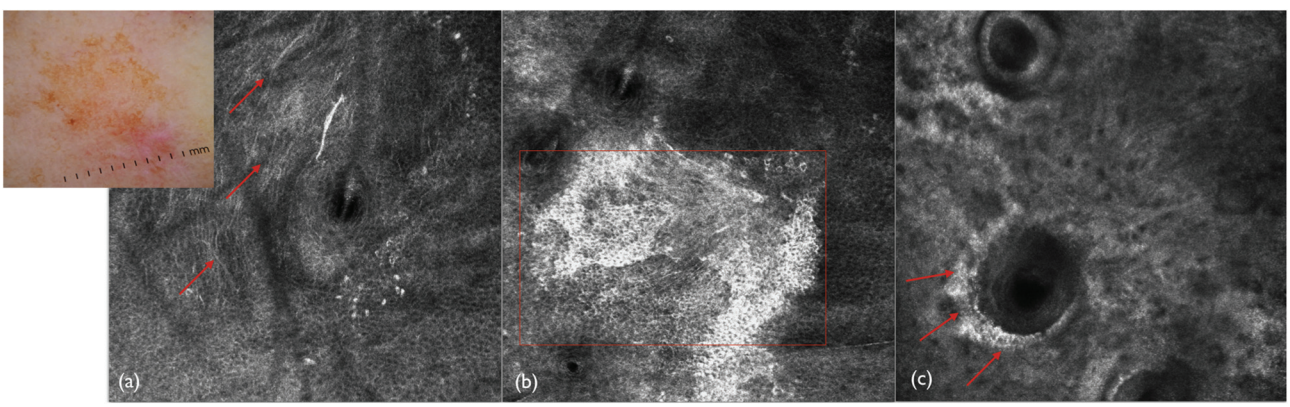

Fig. 1 Pigmented actinic keratosis (PAK). Numerous dendritic cells (arrow) in the area between hair follicles, not infiltrating the infundibulum (a) and irregularly shaped pigmented keratinocytes (rectangle) at the spinous cell layer (b). Pigmented keratinocytes (arrow) in the basal layer of hair follicles (c).

RCM criteria characteristic of solar lentigo/seborrheic keratosis (SL/SK) have been described and are useful for the differential diagnosis with LM and other skin lesions.[6,24,25] The main RCM features of SL/SK are: densely packed edged papillae, polycyclic papillary contour, bright branching tubular structures and bulbous projections at the DEJ.[6,24,25] In addition, multiple plump-bright cells, suggestive of aggregates of melanophages are often found due to partial regression. RCM criteria suggestive of a melanoma are never seen (Fig. 2).

An analysis of differential RCM features for benign lesions and facial LM showed that the most important parameters allowing clear distinction include the absence of pagetoid and atypical cells within the epidermis, along with the presence of a regular honeycombed or cobblestone pattern and ringed or polycyclic contours at the DEJ.[6] Of note, the presence of inflammatory cells in the upper dermis is frequently observed in both benign and malignant lesions, suggesting that the presence of inflammatory cells is not a differential feature.[6]

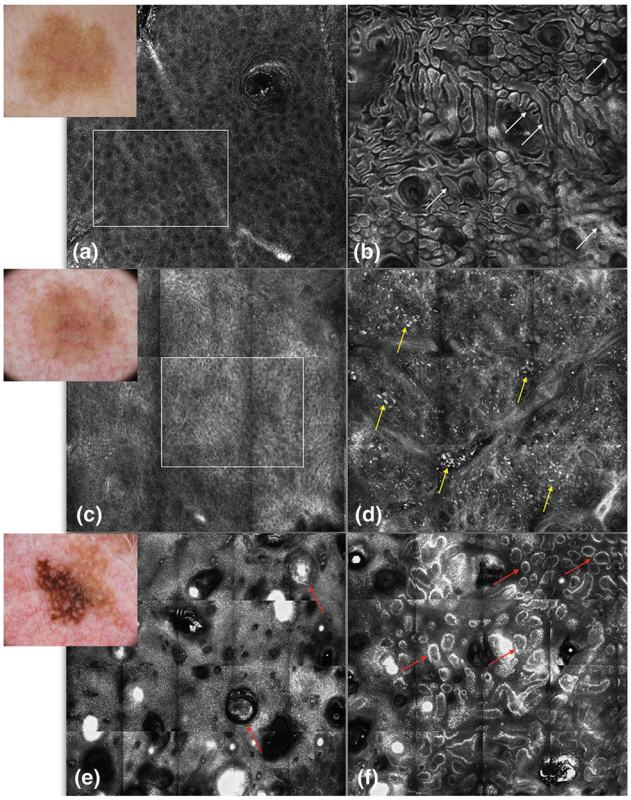

Fig. 2 Solar lentigo (SL) and seborrheic keratosis (SK). The RCM images of a classic solar lentigo of the face, show a normal honeycomb pattern (white rectangle) at the spinous layer (a) and the presence of densely packed edged papillae, bright branching tubular structures and bulbous projections (white arrow) at the dermal epidermal junction (DEJ) (b). RCM images of a lichen planus like keratosis (LPLK), showing a normal honeycomb pattern (white rectangle) at the spinous layer (c) and the presence of multiple plump bright cells (yellow arrow) at the DEJ (d). RCM images of a clonal SK, showing a normal cobblestone pattern with milia like cysts (red arrow) at the spinous layer (e) and the presence of polycyclic papillary contours (red arrow) and bulbous projections at the DEJ (f).

Lentigo maligna

RCM features of LM/LMM have been extensively described.[17,26,27] The main criteria for LM/LMM diagnosis include the presence of numerous round or dendritic pleomorphic cells, distributed in all layers.[7,28] Differential features include the presence of atypical cells grouped around a hair follicle,[7,29] the presence of melanophages and reticulated bright collagen bundles in the papillary dermis due to solar damage. Small nests of atypical and nucleated melanocytes were also described as structures corresponding to globules visualized at dermoscopy.[6,30]

A study of RCM examination revealed that the most relevant pattern associated with LM/LMM was the presence of atypical cells both at the junction and spreading upwards in a pagetoid fashion, usually with a dendritic morphology.[6] An important feature, known as 'Medusahead-like' structures, characterized by elongated buddings bulging from the hair follicle and populated by dendritic/pleomorphic cells, was found frequently in LM/LMM, usually in relation to asymmetric follicular pigmentation and/or around hyperpigmented follicles. Furthermore, epidermal disarrangement and a meshwork pattern was observed in LM/LMM.

Additionally, the arrangement of atypical cells can be characterized by folliculotropism. In a recent study a more precise distribution of pagetoid and dendridic cells in LM/LMM was characterized: the dendridic cells tend to infiltrate perifollicular epidermis and the inner portion of the hair follicles "folliculotropism" and the pagetoids cells tend to bulge around the follicles[13] (Fig. 3).

A combination of the most relevant above mentioned features helps to correctly identify the majority of LM/LMM: melanocytic nests, roundish pagetoid cells, follicular infiltration, bulging from the follicles and many bright dendrides infiltrating the hair follicles.

Differential diagnosis in challenging cases

Preliminary reports showed that RCM could be used to differentiate facial LM/LMM from NMSN.[7,29,31,32] Guitera and colleagues[17] assessed the sensitivity and specificity of 64 RCM features of LM in a series of dermoscopically equivocal facial macules (81 LM and 203 benign) and proposed an LM score to distinguish between the lesions. The score criteria consists of 2 major, non edged papillae and round pagetoid cells, and 4 minor criteria, including ≥3 atypical cells at the DEJ in five 5 mm × 5 mm^2 fields, follicular localization of pagetoid cells and or atypical junctional cells, nucleated cells within the dermal papillae and broadened honeycomb pattern. The criteria are outlined in Table 1. An LM score greater than 2 resulted in a sensitivity of 85% and specificity of 76% for a correct LM diagnosis. The selected criteria were proven useful in the differential diagnosis between LM and NMSK pigmented macules, but in some challenging cases, differential diagnosis can be difficult to achieve.[7,17,31,33] The first scenario is represented by the differential diagnosis derived from an early LM and PAK when dendridic cells in epidermal proliferation

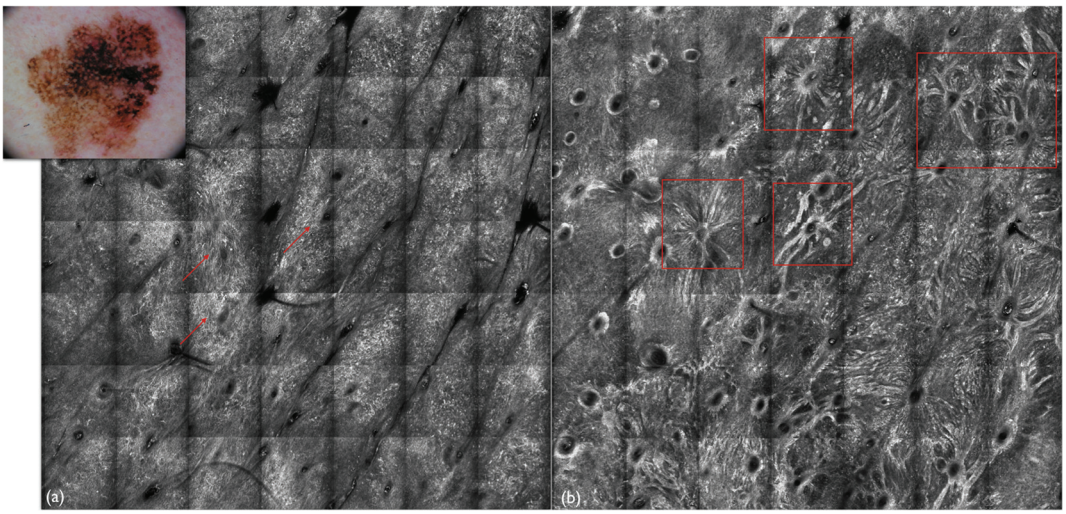

Fig. 3 Lentigo maligna (LM). Pagetoid cells (arrow) with a dendritic morphology at the spinous cell layer (a). 'Medusahead-like' structures (rectangle), characterized by elongated buddings bulging from the hair follicle and populated by dendritic/pleomorphic cells. The arrangement of atypical cells is characterized by folliculotropism, where the dendridic cells tend to infiltrate perifollicular epidermis and the inner portion of the hair follicles (b).

Table 1 LM score, as proposed by Guitera and collegues.[17] Score >1 sensitivity of 93% and specificity of 61% for LM. Score >2 sensitivity of 85% and specificity of 76%

Criteria		Points
Major	Non edged papillae	+2
	Round pagetoid cells >20 μm	+2
Minor	Three or more atypical cells at DEJ in five 0.5 × 0.5 mm^2 fields	+1
	Follicular localization of pagetoid cells and or atypical junctional cells	+1
	Nucleated cells within dermal papillae	+1
	Broadened honeycombed pattern	−1

are present. Nascimento *et al.* recently showed that dendridic cells in the epidermal layer around the hair follicles are frequently present also in PAK, and are generally associated with a regular honeycombed pattern.[14] In a recent study, Persechino *et al.* described the assessment of the dendridic cells including the contour and bulging around the follicle (resulting in the "medusa head like structure" when present throughout the entire follicle perimeter) and the infiltration of dendridic cells in the perifollicular epidermis and in the inner portion of the hair follicle (*i.e.* folliculotropism).[13] The data provided showed that in 68% of LM/LMM the dendridic cells were more abundant and infiltrating the inner portion of the hair follicle infundibulum (*i.e.* folliculotropism), Fig. 4. Further, bulging around the follicles (also called medusa head like structure) was observed in 62%. The differential diagnosis between SL/LPLK and LM/LMM is easier, as SL and LPLK are characterized by regular epidermal layer (either honeycombed or cobblestone) and the absence of round and bright dendrites, well defined follicles and polycyclic papillary contour[34,35] (Table 1). In addition, whenever a pathologic confirmation of the diagnosis is needed, the use of RCM for targeted biopsy of large suspicious lesions on the face appears to be a very useful application of RCM, allowing the reduction of sampling error, helping in the selection process of the best sampling site and avoiding unnecessary biopsies. In the near future, RCM may also be used to assess for LM invasion prior to the decision to treat with non-surgical modalities, although further studies are needed to demonstrate this application.[11,36]

Margin mapping

Although dermoscopy and Wood's lamp have been used to guide LM excisions, their accuracy in the detection of the correct excisional margin of LM/LMM is poor.[36] Recently, two independent studies by Yélamos *et al.* and Pellacani *et al.* demonstrated that the application of the handheld RCM RCM (Vivascope 3000 © Mavig, Munich, Germany) is able to improve LM/LMM margin detection with respect to clinical and dermoscopic examination.[9–11] RCM allows the identification and delineation of subclinical tumor extension. Pre-surgical mapping of the involved area helps guide the clinician and the patient toward the most appropriate management options, assisting surgeons planning reconstruction, and helps the patient understand the size of anticipated postoperative defect if surgery is undertaken. RCM also reduces the risk of positive margins after surgical excision.[9,36,37]

Yélamos *et al.* used an imaging approach that parallels the design of staged excision with radial histopathologic sectioning, by imaging with handheld RCM from the center to the periphery in a radial mode. They estimated the surgical defect area in a series of 23 lesions (19 LM and 4 LMM) of the head and neck prior to staged excision.[10] Navigation was guided by the use of adhesive rings placed along clinical margins. Their results suggested that the margins identified by radial-video-mosaics obtained from handheld RCM images (HRCM-RV),

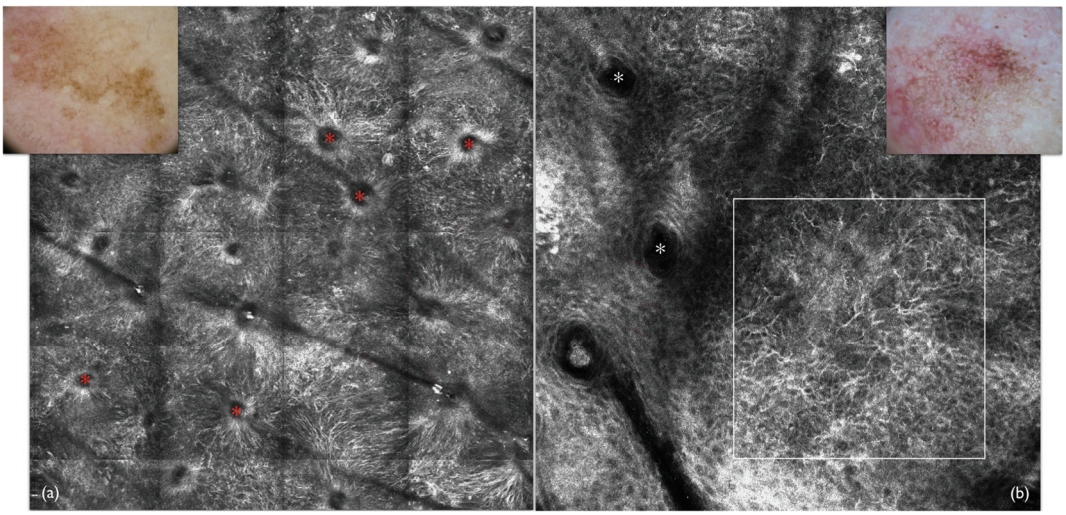

Fig. 4 Comparison between Lentigo maligna (LM) and Pigmented actinic keratosis (PAK). LM is characterized by the presence of folliculotropism, which is the infiltration of dendridic cells in the perifollicular epidermis and in the inner portion of the hair follicle (red asterisks) (a). PAK shows numerous dendritic cells in the area between hair follicles (rectangle), not infiltrating the infundibulum (white asterisks) (b).

correlated well with surgical defects after staged excision of LM/LMM, although the average handheld RCM predictions tended to be smaller than the actual defect, probably because of the difficulty assessing LM edges in a background of sun-damage. HRCM-RV images were obtained using an algorithm written in MATLAB (Mathwork, Natick, MA). Briefly, frames from the handheld RCM recorded video were extracted and then stitched together to create mosaics of the imaged area.[10,11]

Pellacani *et al.* described the superficial margin assessment with hand-held RCM technology (SMART) technique, to perform the margin mapping of LM/LMM.[9] Their study demonstrated that dermoscopically assisted clinical delineation was accurate in only 26% of cases, while RCM-based tumour border delineation was correct in 91% of cases. Margins were evaluated with handheld RCM and annotated using shallow skin cuts, making them clearly identifiable for everyone, including the surgeon. If a margin was positive at 'first-step' RCM evaluation, they sequentially advanced the margin radially outward at that segment by 2 mm intervals until an RCM-negative margin was identified. Using this technique, margin mapping is non-invasive and can be completed preoperatively, so that surgery can be performed in one stage, with high inter-observer agreement.[9]

Margin mapping of LM/LMM with handheld RCM, using superficial skin cuts (SMART) or Video-mosaics, appears feasible and promising however, larger studies are needed to validate these approaches.

Conclusion

RCM proved useful for differential diagnosis for pigmented macules of the face, in particular the diagnostic features for LM have been identified, and include atypical cells both at the DEJ and spreading upwards into the epidermidis with peculiar "folliculotropism", usually with a dendritic morphology. These findings may help to provide a characterization of lesions difficult to diagnose with dermoscopy, due to the absence of distinctive dermoscopy diagnostic features. In particular RCM is useful in differential diagnosis between LM/LMM and other NMSN, such as PAK, where the different distribution of the dendritic cells and follicular infiltration with bulging, and may guide therapeutic management. RCM is also able to assist in determining the precise tumor border in the pre-operative phase, and integrated with Mohs surgery may eventually save the time by reducing the required number of Mohs stages.

Conflicts of interest

There are no conflict of interest to declare.

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