SUPPLEMENTARY MATERIAL

TABLES

Supplementary table 1. Tissue sample investigations based on biopsies.

Analysis and localization of skin tissue	Staining	Evaluation	Parameters, scores
Histopathology			
Whole skin	Hemalun-eosin	• Nature of the inflammatory elements (eosinophilic polynuclear cells, neutrophils, lymphocytes), quantity (rare cells, islet or several scattered foci) • Foreign body reaction with giant cell, number of foci present in the skin • Entire thickness of the dermis • Other local tissue effects	Presence and type of inflammatory cells
Histomorphology			
Dermis	Picrosirius red	• Skin renewal: dermal neocollagenesis	Total collagen content (TCC, %): collagen area/analysed area (10 analysis per slide) at 2 levels: superficial and mid dermis
Dermis	HES	• Skin renewal: dermal thickness	Mean thickness of dermis (15 analysis per slide) measured between dermo-epidermal junction and hypodermis
Whole skin	Alcian blue	• Semi-quantitative assessment of GAGs (HA pertains to this category). • Quantitative analysis by morphometric analysis of elastic fibres.	• Intensity scoring at epidermal and dermal levels from 0 to 4 for GAGs (negative to very significant) for each stain. • % of elastic fibres in superficial dermal: 4 analyses per slide
Immunohistology			
Epidermis	Ki67 antibody with immunodetection kit (DAKO), revealed by AEC	• Skin renewal: epithelial proliferation, by staining of epithelial cells in the M, S, G1 and G2 phase of the cellular cycle.	% of stained cells / total basal cells on a defined area
Epidermis	NCL-CD44-2 (Novocastra), monoclonal antibody to CD44, keratinocyte receptor of hyaluronic acid (in relation to epidermis hydration), with CSA kit (DAKO), revealed by AEC	• Epidermal hydration: revealed by presence of hyaluronic acid receptor. • Analysis of the entire histological section	Staining intensity *Score 0: no staining* *Score 1: low intensity* *Score 2: medium* *Score 3: high* *Score 4: very high* Staining topography *Score 0: no staining* *Score 1: staining of 1/3 of epithelium thickness* *Score 2: staining of 2/3 of epithelium thickness* *Score 3: staining of whole epithelium thickness*
Dermo-epidermal junction	Collagen IV antibody (PHM-12 monoclonal antibody), with ABC kit (Vector Labs), revealed by AEC	• Collagen IV at the dermo-epidermal junction, using PHM-12 antibody. • Analysis of the entire histological section	Staining intensity *Score 0: no staining* *Score 1: low intensity* *Score 2: medium* *Score 3: high* *Score 4: very high*

Supplementary table 2. Subject demographics and baseline characteristics

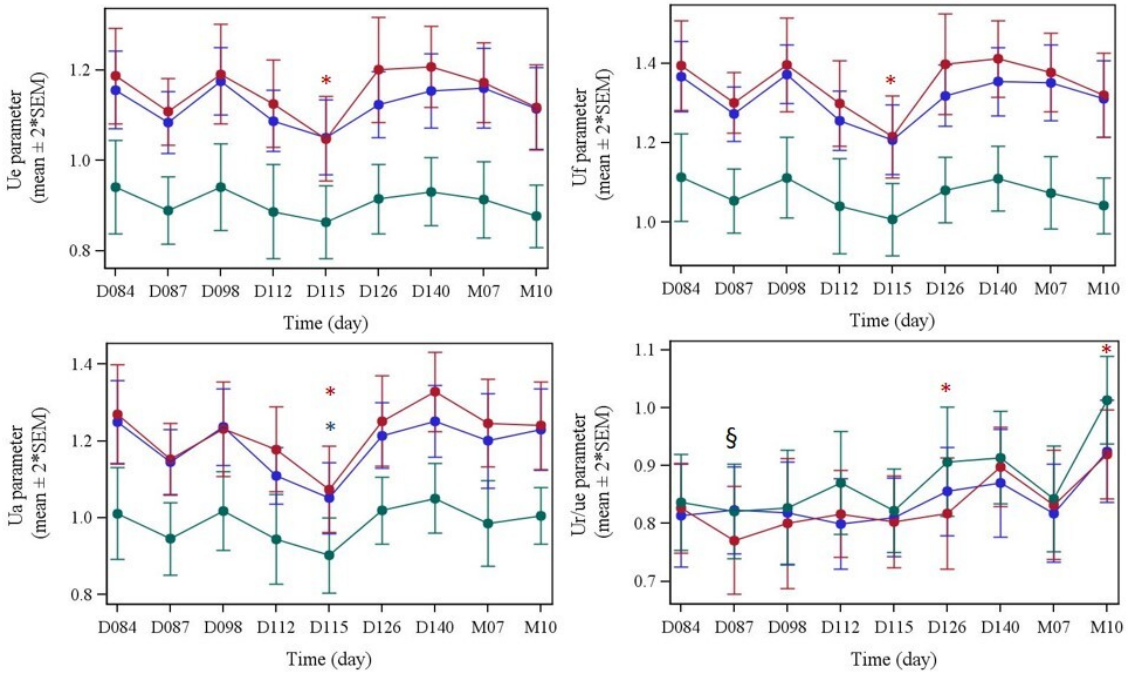
Variable	Total
Age, years (*n*=22)	
Mean (SD)	54.3 (±7.5)
Range	43.0-65.0
Sex, *n* (%) (*n*=22)	
Male	3 (13.6%)
Female	19 (86.4%)
Ethnicity, *n* (%) (*n*=22)	
Caucasian	22 (100%)
African	0
Metis	0
Indian	0
Asian	0
Woman with childbearing potential, *n* (%) (*n*=19)	
Yes	5 (26.3%)
No	14 (73.7%)
Reason for non-childbearing potential, *n* (%) (*n*=14)	
Menopause	13 (92.9%)
Hysterectomy or bilateral ovariectomy	1 (7.1%)
Serology, *n* (%) (*n*=22)	
Positive	0
Negative	22 (100%)
Pregnancy test results, *n* (%) (*n*=5)	
Positive	0
Negative	5 (100.0%)

SD: standard deviation

Supplementary table 3. Injection site reactions (ISRs) in the neckline

	Immediately after the first injection (D84)		Immediately after the second injection (D112)	
Number of subjects with:	**KIO015 *n*=21**	**HA *n*=21**	**KIO015 *n*=20**	**HA *n*=20**
Erythema	18 (86%)	19 (90%)	7 (35%)	7 (35%)
Pain	5 (24%)	1 (5%)	0 (0%)	0 (0%)
Induration	21 (100%)	21 (100%)	20 (100%)	20 (100%)
Swelling	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Lumps	21 (100%)	21 (100%)	20 (100%)	20 (100%)
Hematoma	2 (10%)	5 (24%)	0 (0%)	4 (20%)
Itching	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pigmentation	0 (0%)	0 (0%)	0 (0%)	0 (0%)

FIGURES

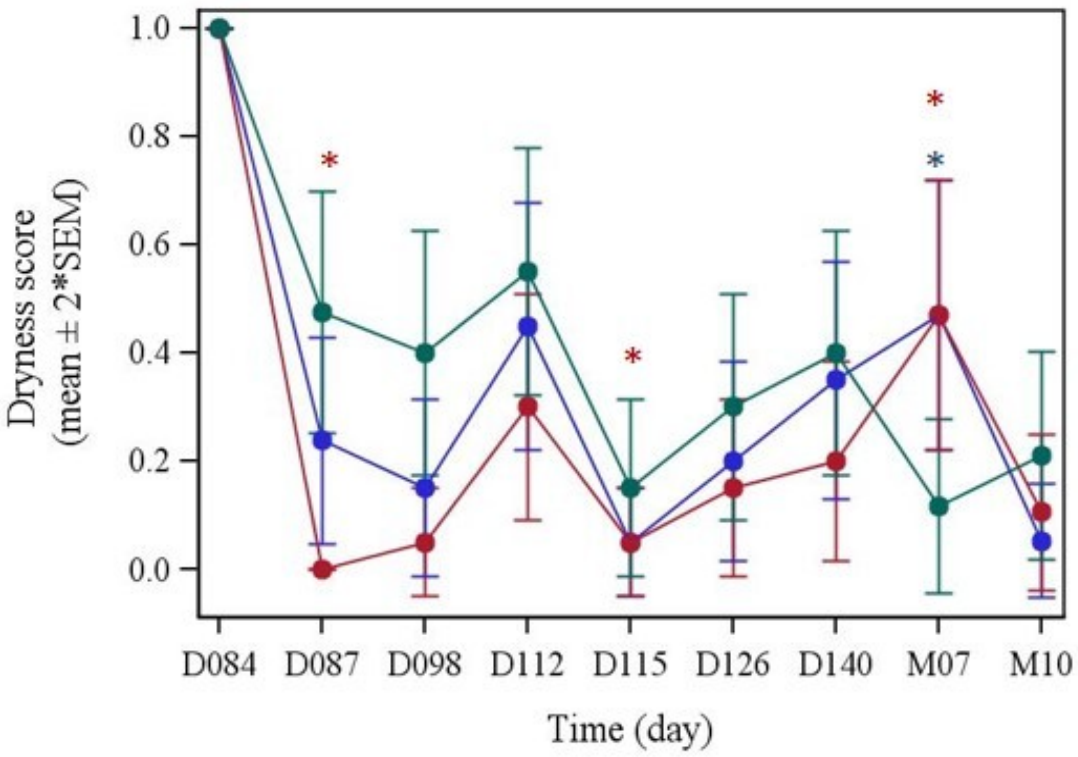


Supplementary figure 1. Mean Cutometer® values over time.

Blue line: KIO015; red line: HA; green line: Untreated zone.

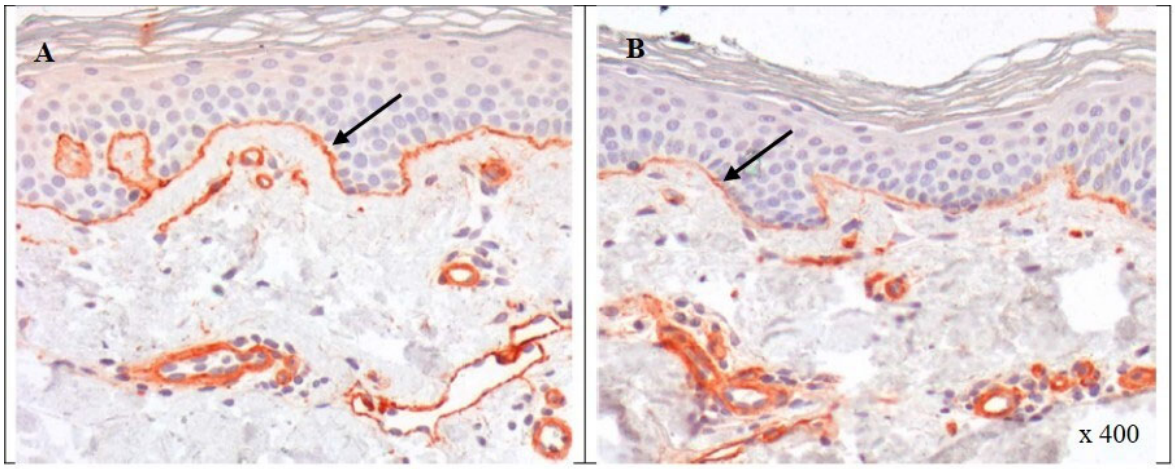
** p<0.05 comparison with untreated zone for change from baseline at each time point.*

§ p<0.05 comparison between KIO015 and HA for change from baseline at each time point.



Supplementary figure 2. Skin dryness score over time.

The investigator scored skin dryness in the neckline on a scale from 0=normal skin to 4=extremely dry skin.
Blue line: KIO015; red line: HA; green line: Untreated zone
** p<0.05, comparison to the untreated zone for change from baseline at each time point. No statistical difference between KIO015 and HA.*



Supplementary figure 3. Collagen IV expression in the epidermal-dermal junction in one subject.

Collagen IV in the dermo-epidermal junction was revealed by immunohistochemical labelling (ABC kit, Vector Labs) using PHM-12 antibody (mouse) and 3-amino-9-ethylcarbazole (AEC). A: KIO015 injected zone; B: HA injected zone.

KIO015 – 1 week	HA – 1 week	Untreated dermis
KIO015 – 4 weeks	HA – 4 weeks	
KIO015- 12 weeks	HA – 12 weeks	

Supplementary figure 4. Dermal collagen expression in rabbits.

Histomorphometric analysis (x400) after Picrosirius red staining 1, 4 and 12 weeks after injection of KIO015 and HA (Belotero® Hydro).