Leids Universitair Medisch Centrum

3D-organotypic skin cultures for research and screening purposes

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Leiden Drug Development Conference
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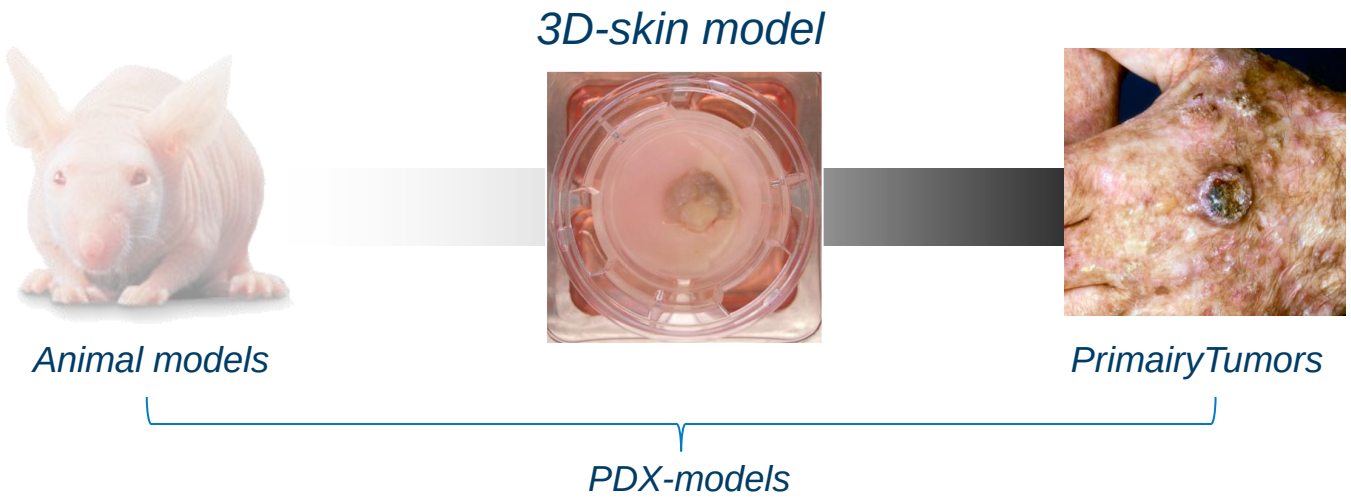
LEIDEN DRUG DEVELOPMENT CONFERENCE

Need for models in dermatological research

- Within dermatology, there is an unmet clinical need to develop therapies for a wide range of skin diseases, including skin cancers (eg, squamous cell carcinoma, melanoma), eczema and psoriasis.



Mission and focus

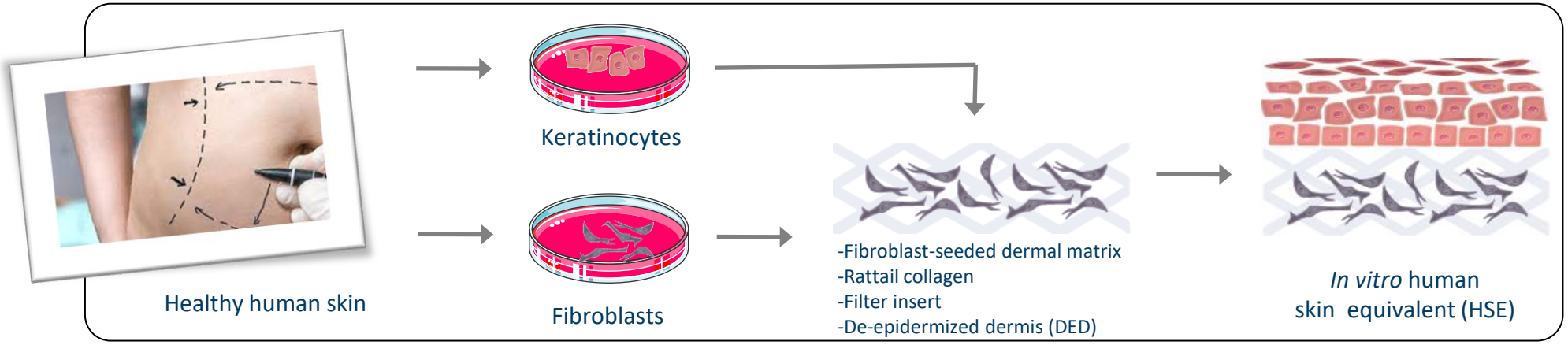
We generate **healthy and diseased** *in vitro* human skin equivalents (HSEs) as tools to study and modulate biological mechanisms in human skin.

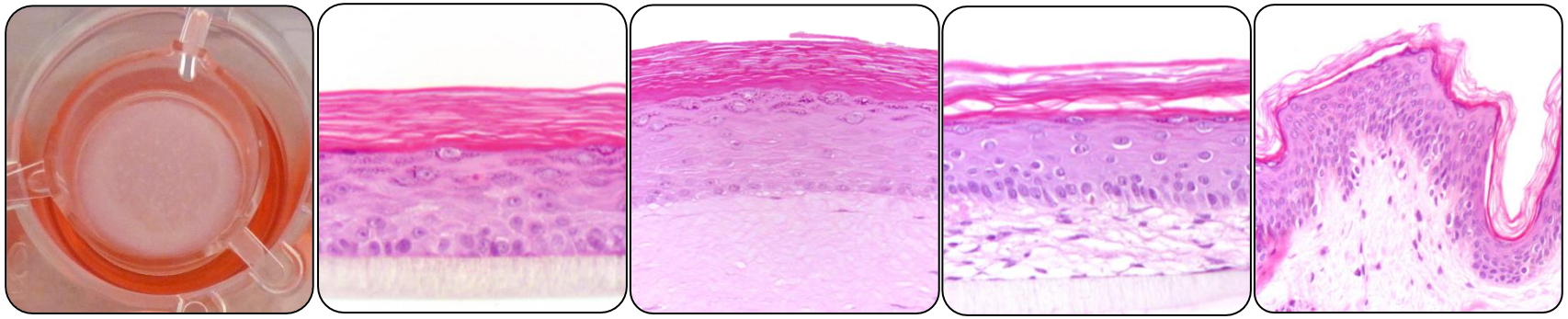
Our mission is to empower the discovery, development, production and marketing of new skin products and regenerative solutions for humans in order to **restore and improve healthy skin**. To this end, we are dedicated to provide stable, reliable, uniform, representative and customizable *in vitro* human skin models that are subject to continuous improvement.

- **Fundamental Research**
- **Compound screening**
- **Co-development**
- **Regenerative medicine**

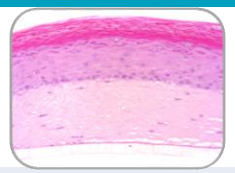
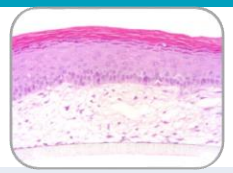
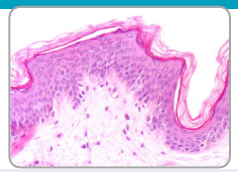
6, 12 and 24 format

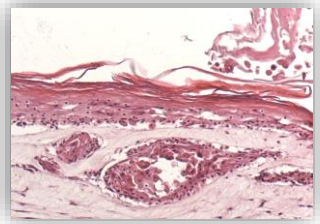
In vitro 3D-human skin equivalent (HSE)



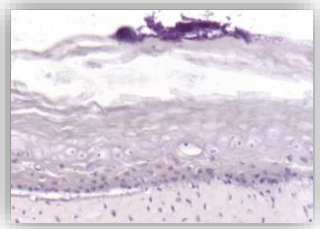


Applications of human skin equivalents

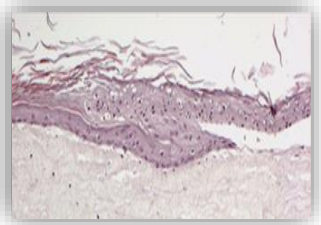
	Leiden Epidermal Model (LEM)	Full Thickness Model (FTM)	Fibroblast derived matrix model (FDM)	Native human skin (ex-vivo)
Morphology				
Cell types	• Keratinocytes • Melanocytes	• Keratinocytes • Melanocytes • Fibroblasts subtypes • Endothelial cells • Macrophages/ T cells • Rattail collagen	• Keratinocytes • Melanocytes • Fibroblasts subtypes • Endothelial cells • Human collagen	• Keratinocytes • Melanocytes • Fibroblasts subtypes • Endothelial cells • Immune cells • Human collagen
Applications	• Toxicity testing • Pigmentation • Penetration • Disease models e.g. - Bacterial skin infection - Fungal skin infection - Wound healing - etc.	• Predictive screening • Disease models e.g. - Squamous cell carcinoma - Melanoma - Head/neck cancer - Eczema - Psoriasis - Monogenic diseases - etc.	• Predictive screening and research on dermal processes e.g. - Wound healing - Skin Aging - Scar formation - etc.	• Short term screening and research on dermal processes e.g. - Wound healing - Bacterial infection - etc.
Format	• 6-well (4,76 cm^2) • 12-well (1,12 cm^2) • 24-well (0,33 cm^2)	• 6-well • 12-well	• 6-well • 12-well	• 6-well • 12-well • Biopsy size (4, 8 mm)
Longevity	• Up to 4 weeks	• Up to 8 weeks	• Up to 20 weeks	• Up to 1 week



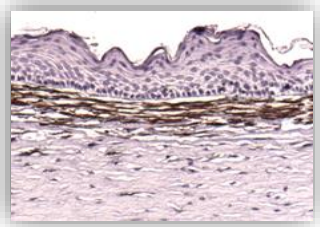
Skin cancers



Microbiome (infection)



Wound healing

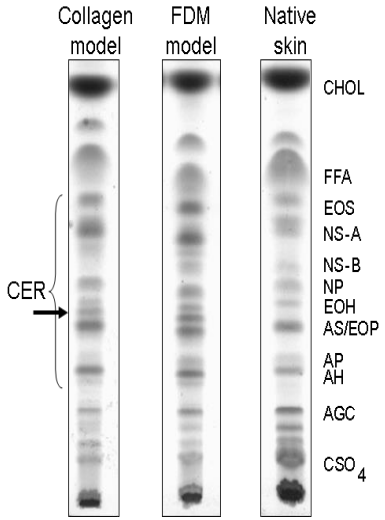


Scar

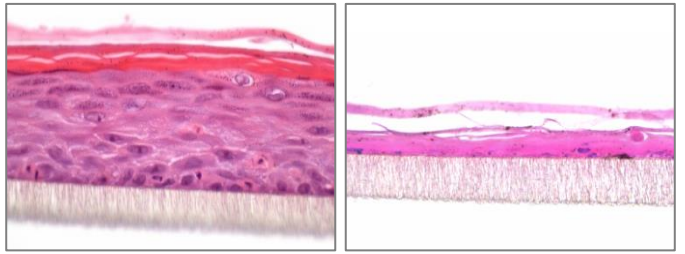
Safety testing according to EU Guidelines

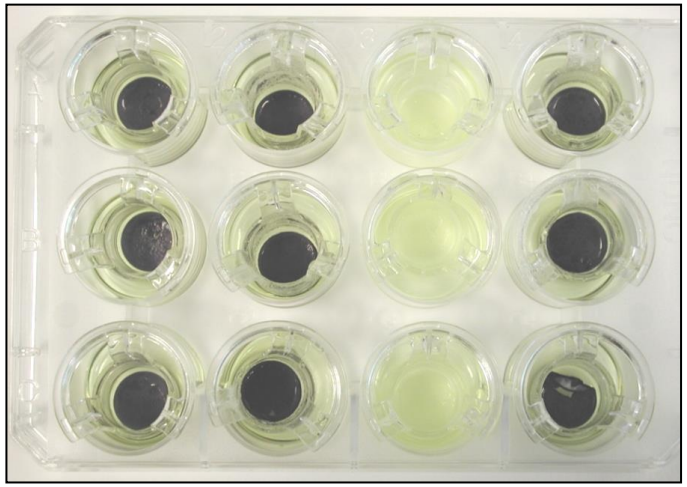
HSEs with competent skin barrier

Lipid composition



	C = Corrosive; NC = Non-corrosive		Corrosivity prediction				
		In vivo		EpiDerm		EpiSkin	
#	Chemical name		LUMC	Unilever	ZEBET	Unilever	ZEBET
1	1,2-Diaminopropane (99%) Acros Organics	C	C	C	C	C	C
2	Acrylic Acid (99.5%) Acros Organics	C	C	C		C	
3	2-tert. Butylphenol (99%) Lancaster Synthesis	C	C	C	C	C	C
4	Potassium hydroxide (10% aq)	C	C	C	C	C	C
5	Sulfuric acid (10% wt.) H_2SO4	C	C	C	C	C	C
6	Octanoic acid (caprylic acid) (98%). Lancaster Synthesis	C	C	C	C	C	C
7	4-Amino-1,2,4-triazole (99%) Acros Organics	NC	NC	NC	NC	NC	NC
8	Eugenol (>95%) ICN Biomedicals	NC	NC	NC	NC	NC	NC
9	Phenethyl bromide (98%) Acros Organics	NC	NC	NC	NC	NC	NC
10	Tetrachloroethylene (99%) Acros Organics	NC	NC	NC	NC	NC	NC
11	Isostearic acid (>95%) ICN Biomedicals	NC	NC	NC	NC	NC	NC
12	4-(Methylthio)-benzaldehyde (98%) Alfa Aecar	NC	NC	NC	NC	NC	NC





EURL-ECVAM

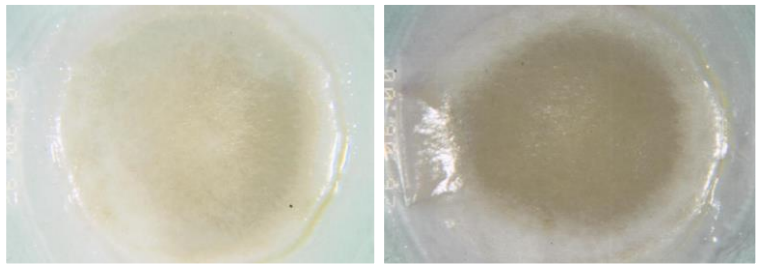
EU validation for skin corrosion and skin irritation:
Replacing the Draize rabbit eye-test

El Ghalbzouri et al., 2008, Thakoersing et al., 2014, 2015,Mieremet et al., 2018, 2019a, 2019b; Helder et al., 2019, 2020, 2021

Testing of pigment-modulating agents

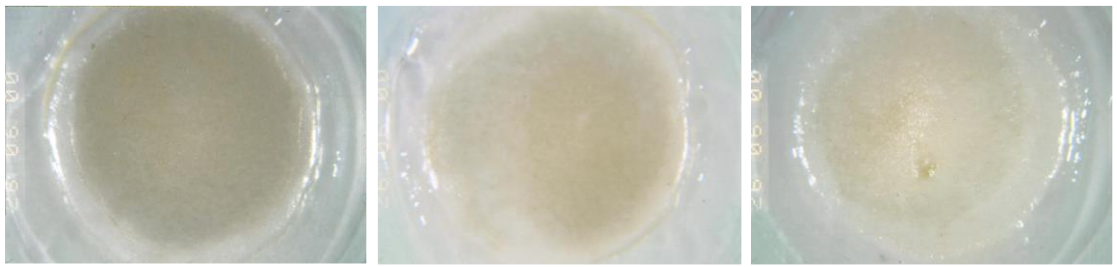
www.Kyraclinic.com

Control 'tanning'



Phototan

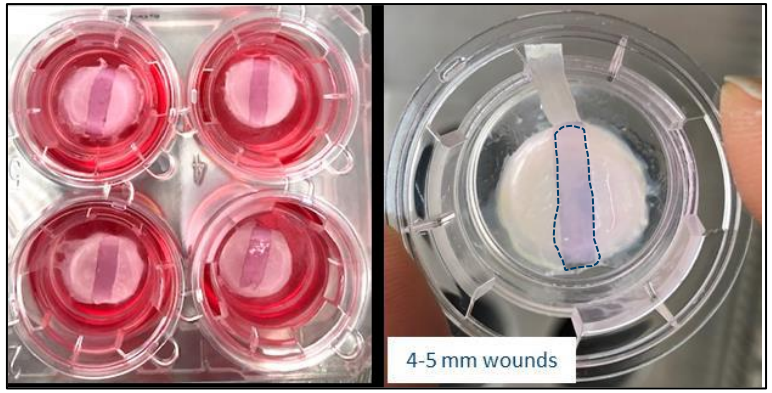
Control 'bleeching'

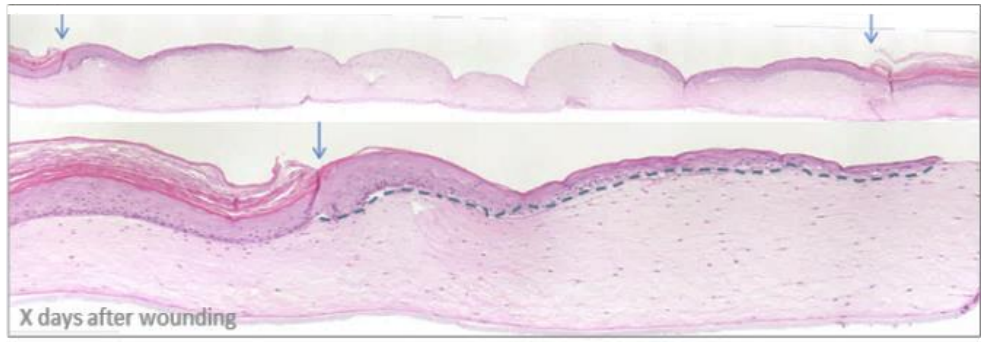


Hydroquinone Kojic acid

Effect of Tyrosur on wound healing in infected wounded HSEs

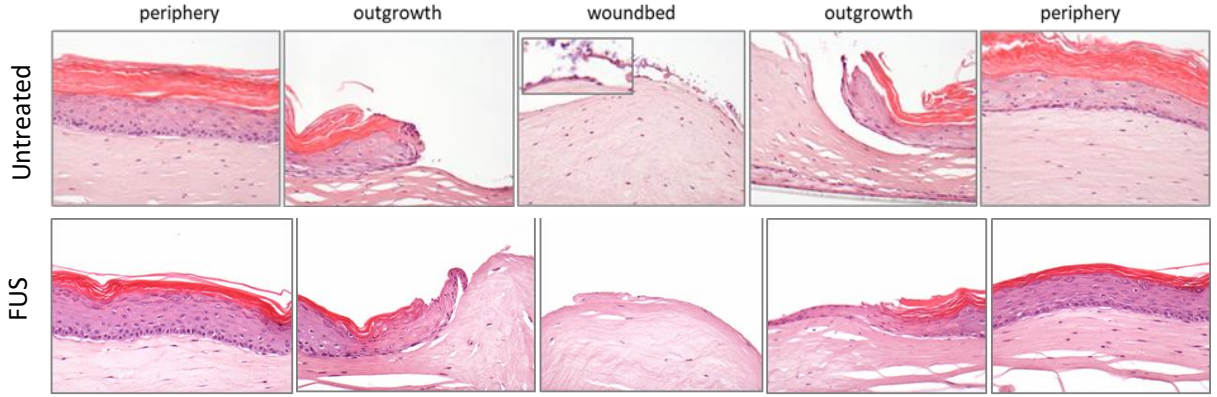
3D-Full-thickness model

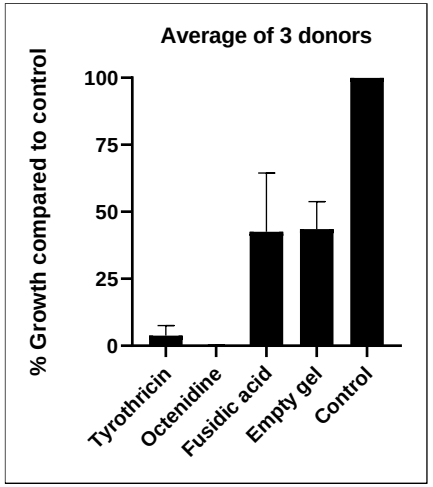




+ (Infection for X hrs with)

- *MRSA,*
- *Staphylococcus epidermidis*
- *Streptococcus pyogenes*

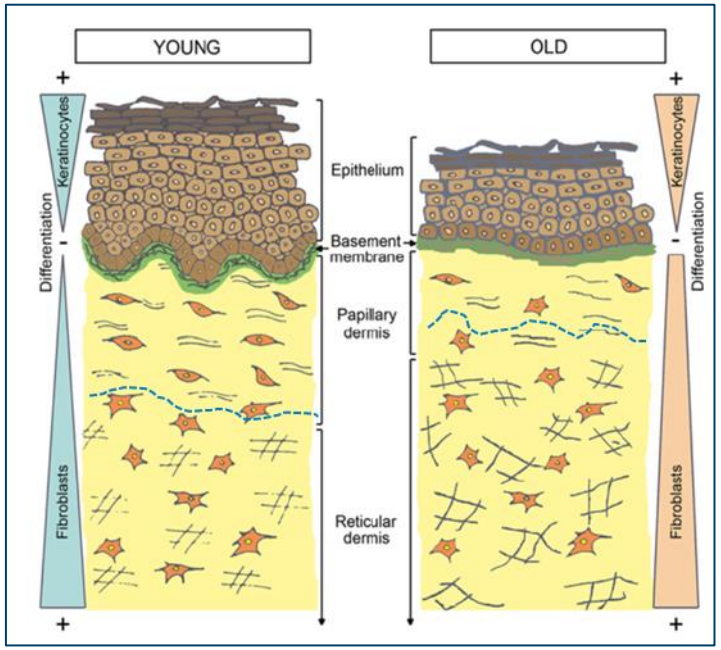


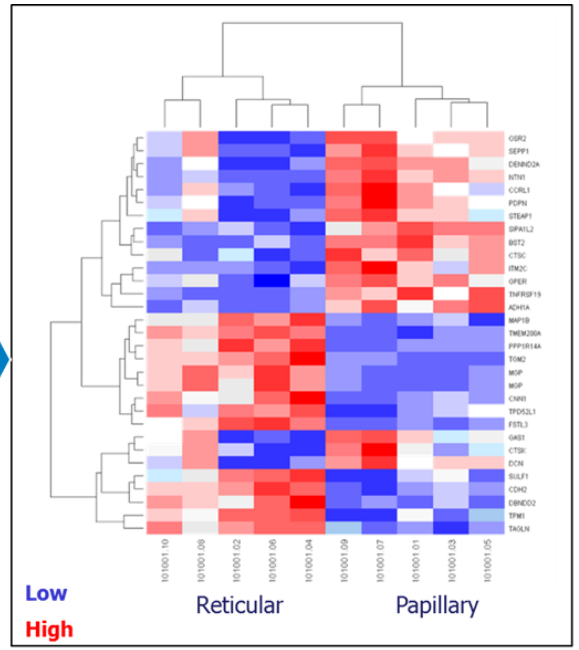


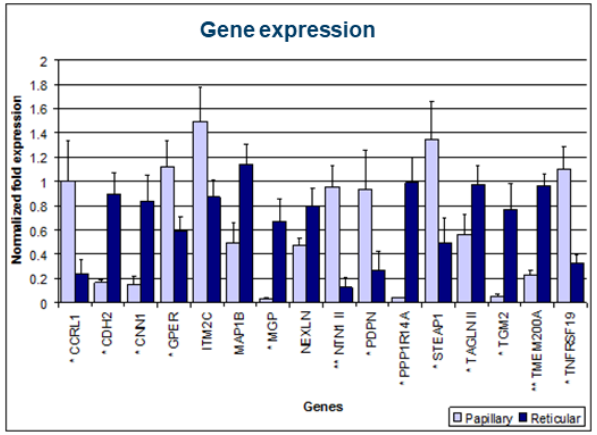
Sponsor: Engelhard Pharma (2017-ongoing) (El Ghalbzouri et al., 2022 in prep preparation)

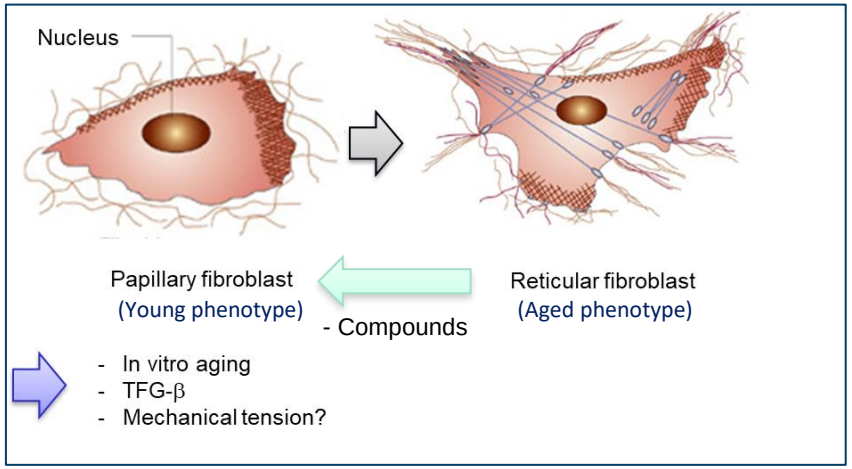
Development of novel anti-aging concept: alteration of the dermal cell population

CHANEL
RECHERCHE ET TECHNOLOGIE
RESEARCH AND TECHNOLOGY

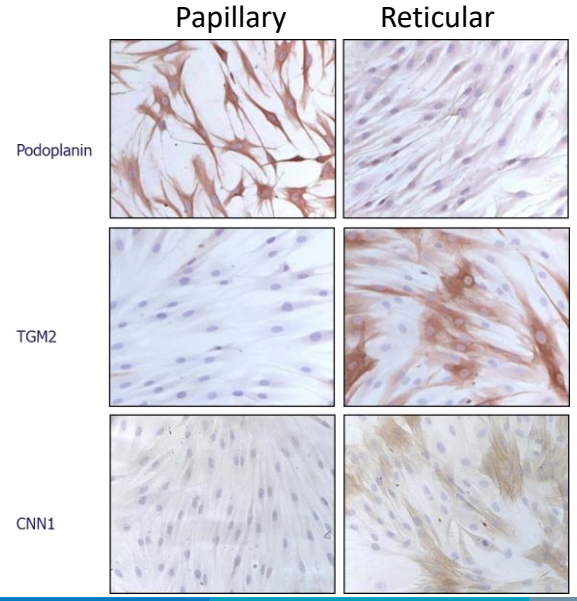






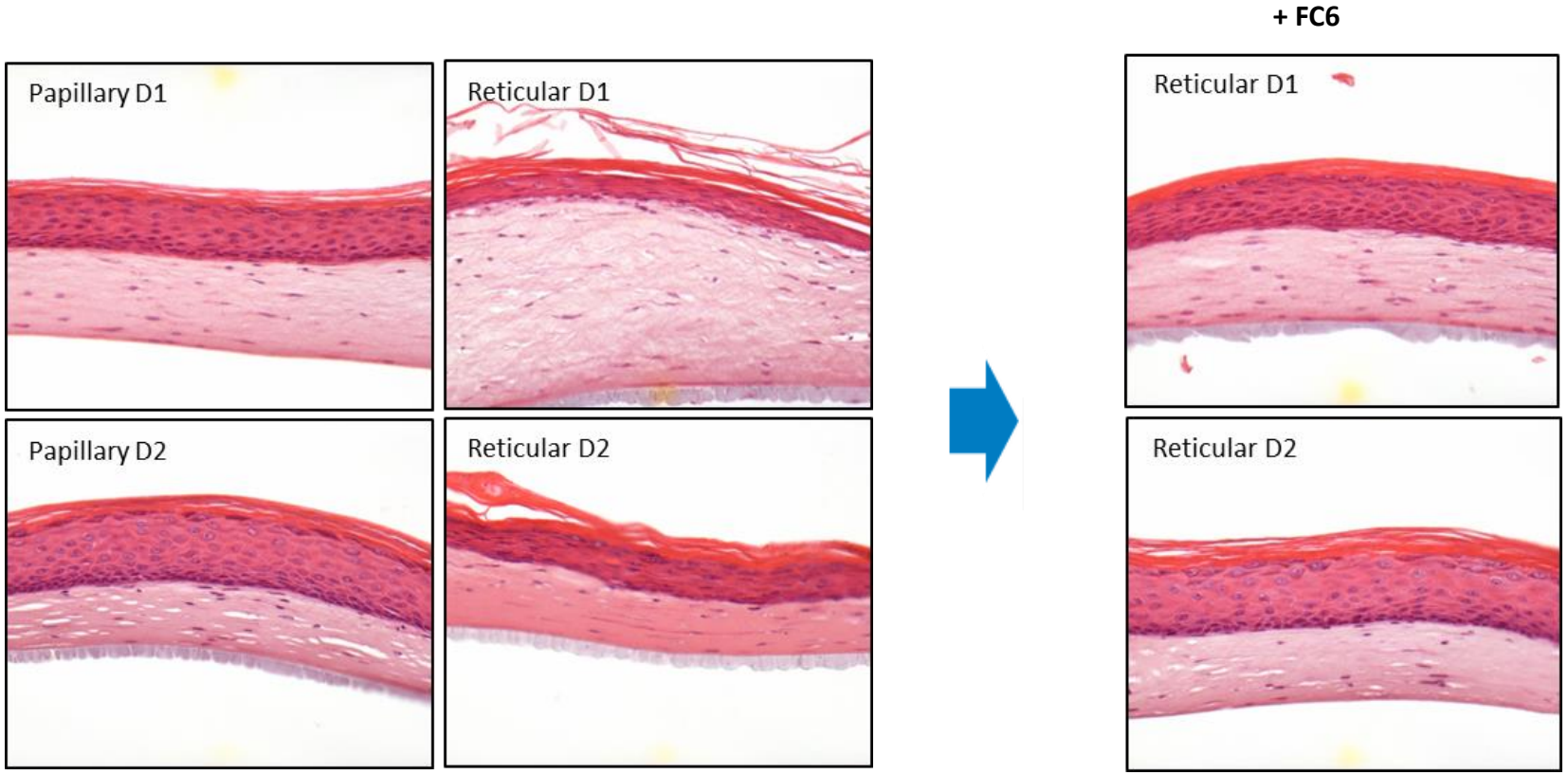


New strategy for skin aging by reverting reticular gene signature in order to correct skin aging phenotype



Sponsor: Chanel Parfum Beauté (2009-ongoing)
Janson et al; 2012, 2013, 2014, 2015

Effect of compounds on Col11A1 expression (FC 6)



Patent: EP20110306566 20111125; WO2012EP73458 20121123

Dissect the heterogeneity of fibroblast subtypes

To identify the origin and define the subsets of CAFs, we aim to dissect the heterogeneity of these fibroblast subtypes, study the differentiation process, and unravel the mechanisms by which cancer cells reprogram these fibroblast subtypes. Finally, we will investigate to what extent ECM produced by Pfs, Rfs or CAFs contributes to tumour invasion and EMT.

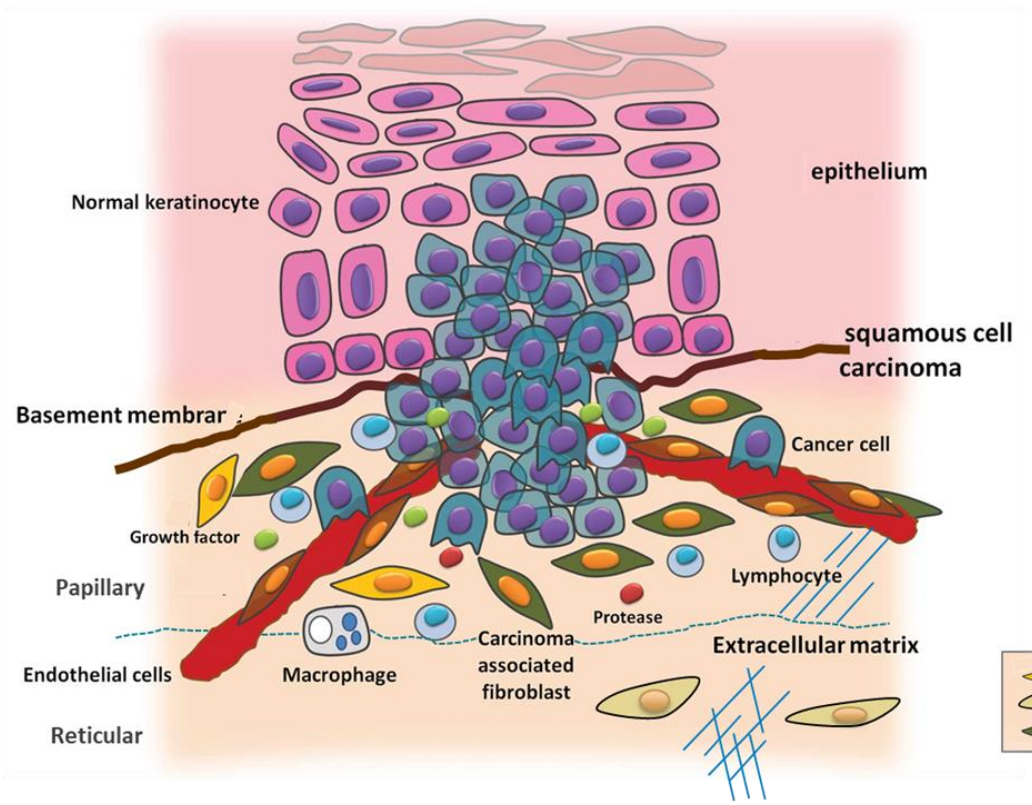
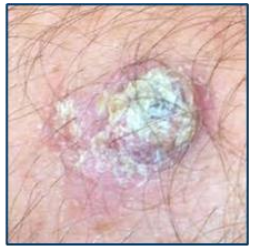
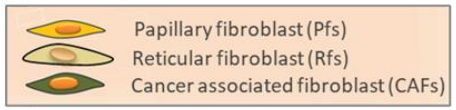


Figure 1 | Schematic illustration of a skin tumor demonstrating the presence of different cell types

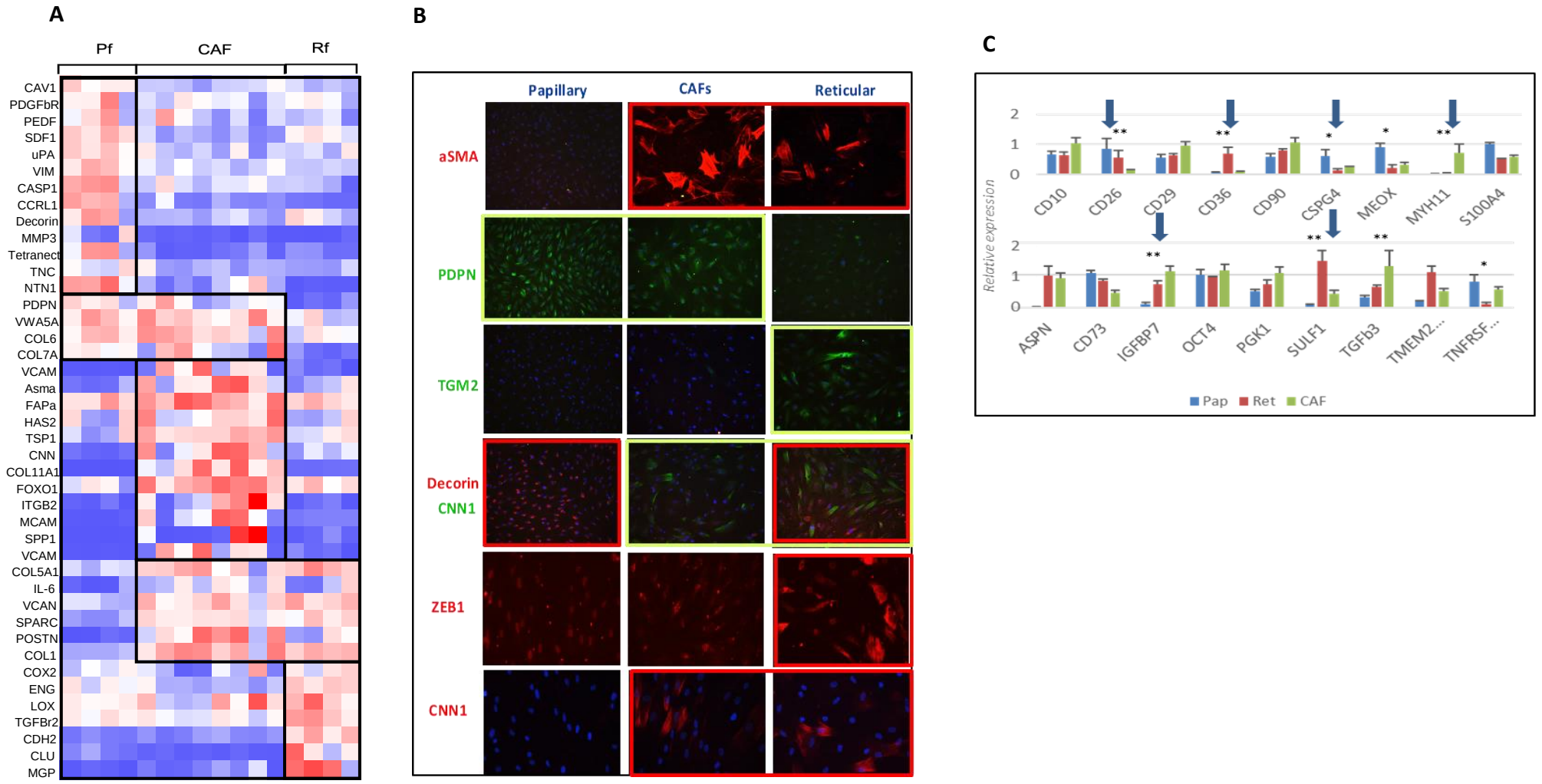


cSCC

Melanoma

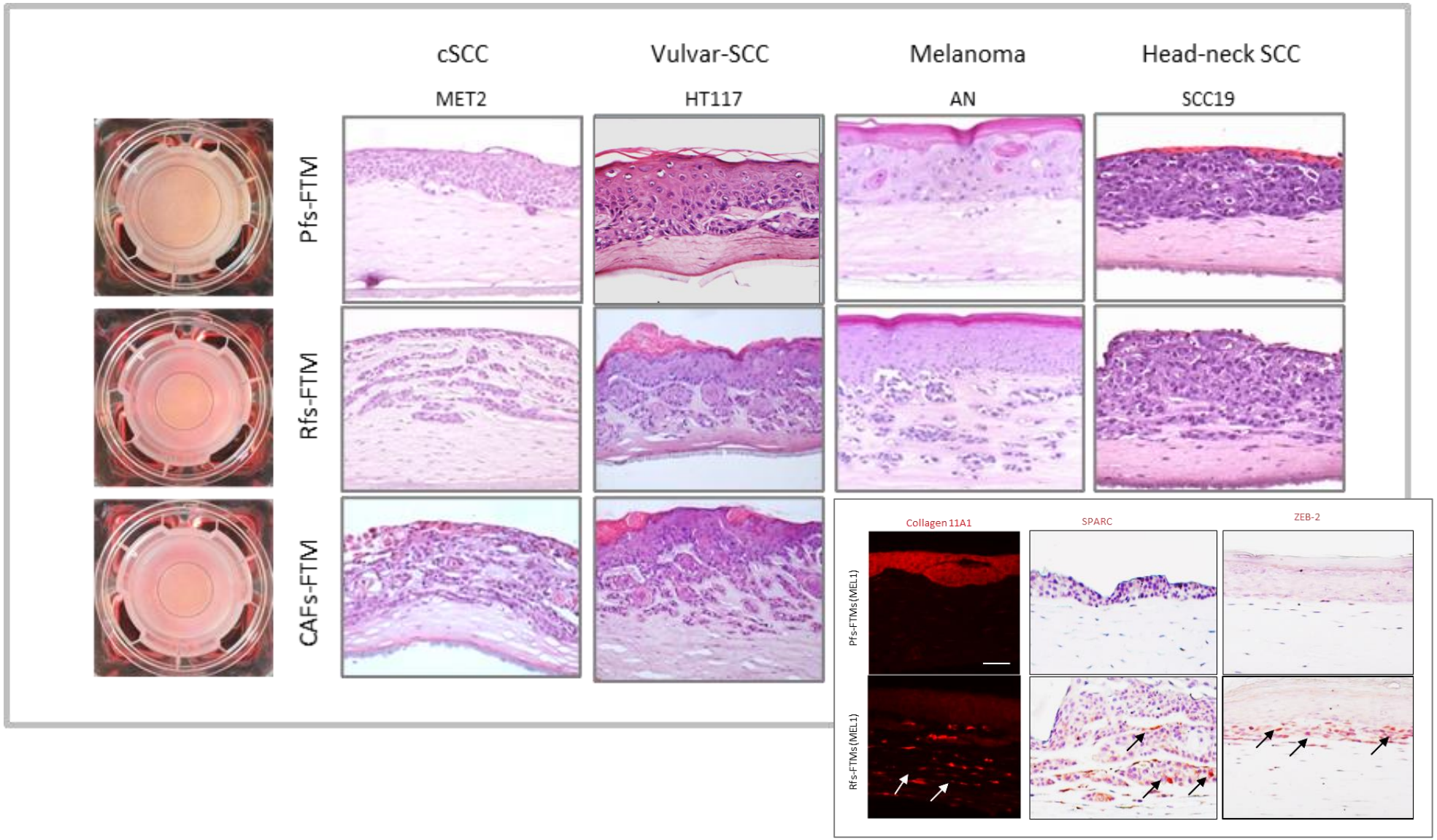


Biomarkers of different fibroblast sub-populations



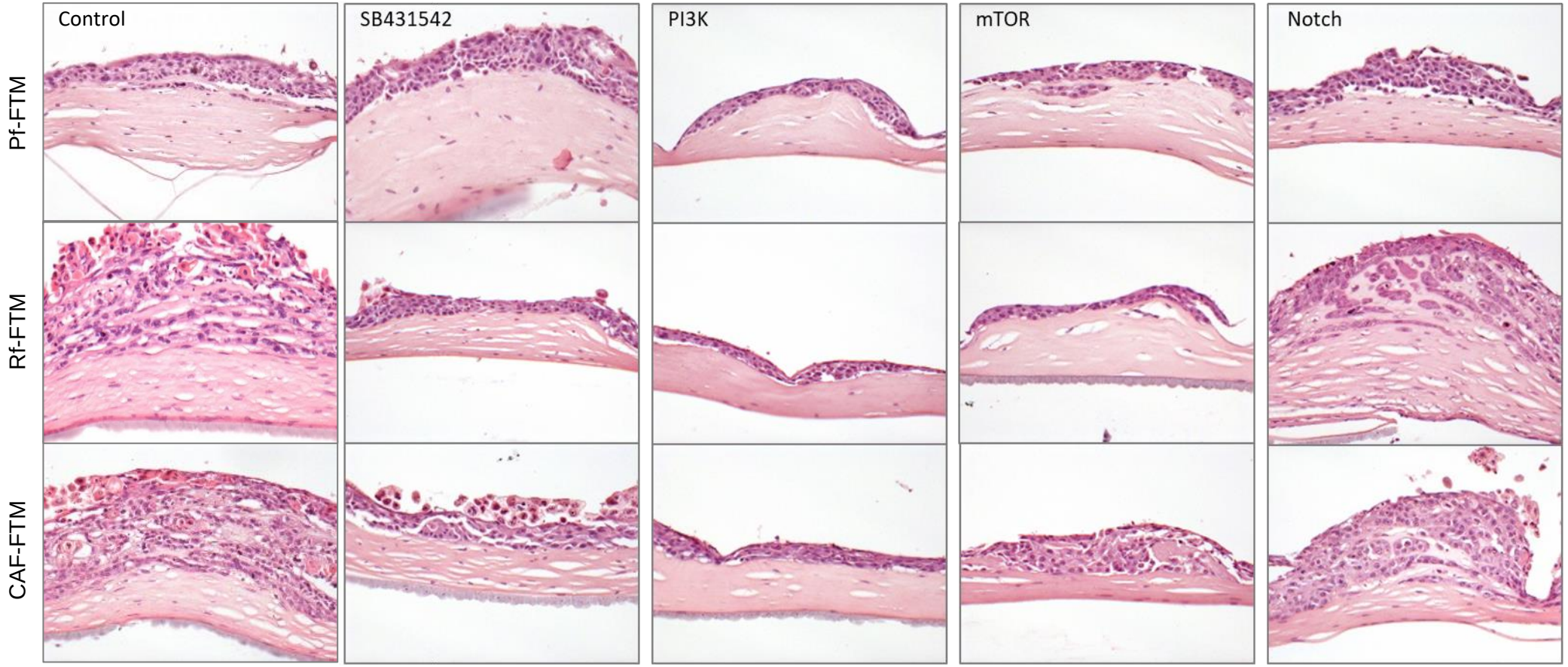
(Janson er al., 2014; 2015, Hogervorst et al., 2018; Wu et al., in prep)

Reticular fibroblasts and CAFs induce invasive behavior of different cancers



Hogervorst et al., 2018, Wu et al., 2022, Huisman et al. 2022;, in preparation

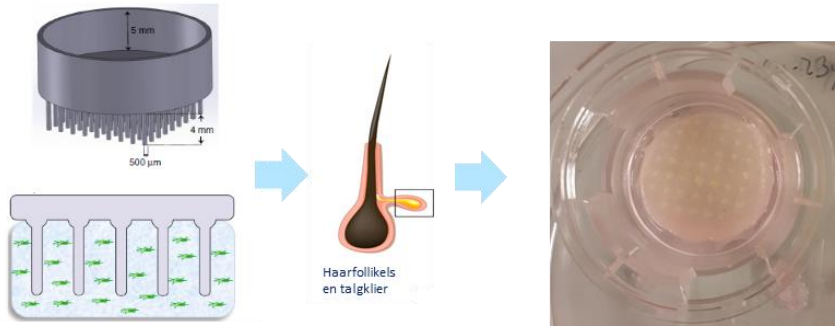
Effect of drug compounds on the invasive behavior of fibroblast subtypes (SCC-cell line)

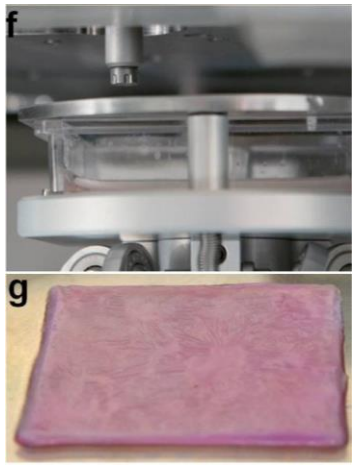


Wu et al. 2022;, in preparation

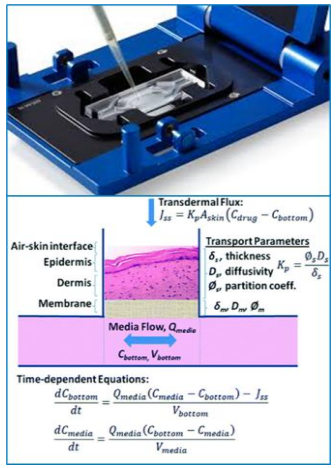
Ongoing developments for innovative alternatives

- → **Tumor micro-environment (e.g. cSCC, melanoma, HNSCC, Vulvar SCC)**
- → **Develop a competent microbiome environment**
- → **Develop skin model for Acne Vulgaris**
- → **Develop various 3D-tissue models (e.g. vulvar, rectal, vaginal)**
- → **Development of immune-competent skin models for healthy and diseased skin**
  - → Keratinocytes V
  - → Melanocytes V
  - → Fibroblasts (subtypes) V
  - → Endothelial cells V
  - → Adipose tissue V
  - → Macrophages (M1/M2) V
  - → Immune cells V
  - → Hair follicles V





3D-Bioprinting

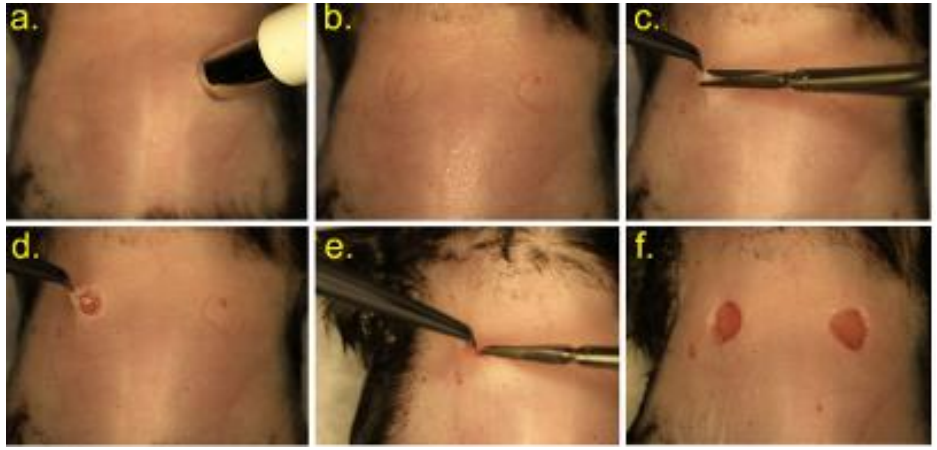


Skin (Organ) on a chip

Reduction and replacement of animals

- Application of drugs through the skin
- Testing of compounds from the chemical, pharmaceutical and food industry on skin penetration, corrosion and irritancy:

Mice	7.114
Rats	74.836
Rabbits	6.922
Pigs	1.263



Wound animal model

Research

- Skin cancer (Melanoma, SCC)
- Wound healing
- Genetic skin diseases
- Eczema
- Angiogenesis
- Psoriasis
- Etc.

Total number of animals used in NL (448,798)

Source: Zo doende 2020

Mice:	148.291
Rats:	87.169
Pigs:	9.192

Thank you!

LUMC Bontius Stichting | LUMC Research Foundation

CHDR Centre for Human Drug Research

Pfizer

GALDERMA

ENGELHARD Arzneimittel

Beiersdorf

Daphne Textiles

InDex Pharmaceuticals

reckitt

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TNO innovation for life

ZonMw

LACDR