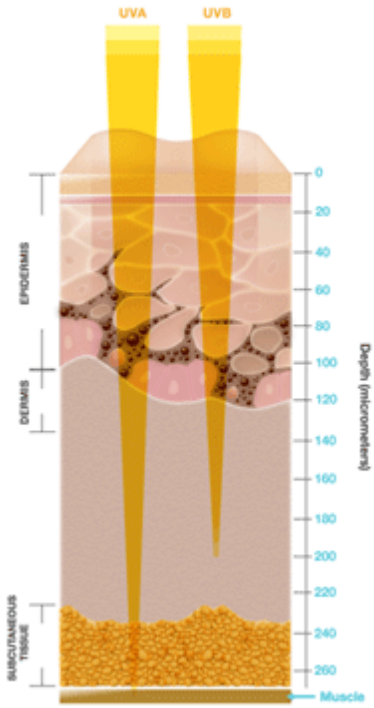
TECHNOLOGY UPDATE I

CLINUVEL

ORIGINAL THOUGHTS ON UV AND PIGMENTATION



UV light penetrates melanocompromised skin and causes damage.

During the late eighties, various scientists simultaneously worked on and developed a number of analogues of alpha-melanocyte stimulating hormone (α-MSH) to study the effect of these molecules on dermal UV-protection. As a matter of fact, the Arizona group initially received funding from the NIH specifically to develop melanoma prevention strategies.

It was well documented that physiological α-MSH played an important role in the natural pigmentary response following UVA and UVB skin damage. A common misunderstanding was that our skin's pigmentation, melanin, served as a facultative layer to provide a beneficial glow after and following sun exposure. The tanning response is, in reality, a physiological repair mechanism to instant UV damage of the skin cells (epidermis/dermis). Further, it was contemplated whether UVB alone was responsible for the long-term mutations seen in the epidermis. It has since been established from carcinogenic models that UVA plays a synergistic and complementary role to UVB, whereby the wavelengths determine the ability to penetrate the skin layers.

Further research in biophysics, both in mammals and humans, demonstrated that the pigmentary response is a cellular reaction to photodamage. The intracellular damage comes in various forms:

- cyclobutane (5-5)
- pyrimidine dimers (CPD)
- pyrimidine pyrimidinone dimers (6-4 PDs)

To explain in greater depth, UV radiation is cytotoxic to all levels of the skin (pandermal), and the prevalent cyclobutane pyrimidine dimers cause photoisomerisation of mainly 6-4 PDs. Further chemical adducts to the UV lesions may include N-acetoxy-N-acteyl aminofluorene (AAAF), benzo(a)pyrene, aflatoxin, photoactivated psoralens and cis-platinum. UVA (320-400nm) is reported to affect all dermal chromophores (light absorbing molecules) which release free radicals to cause DNA strand breaks.

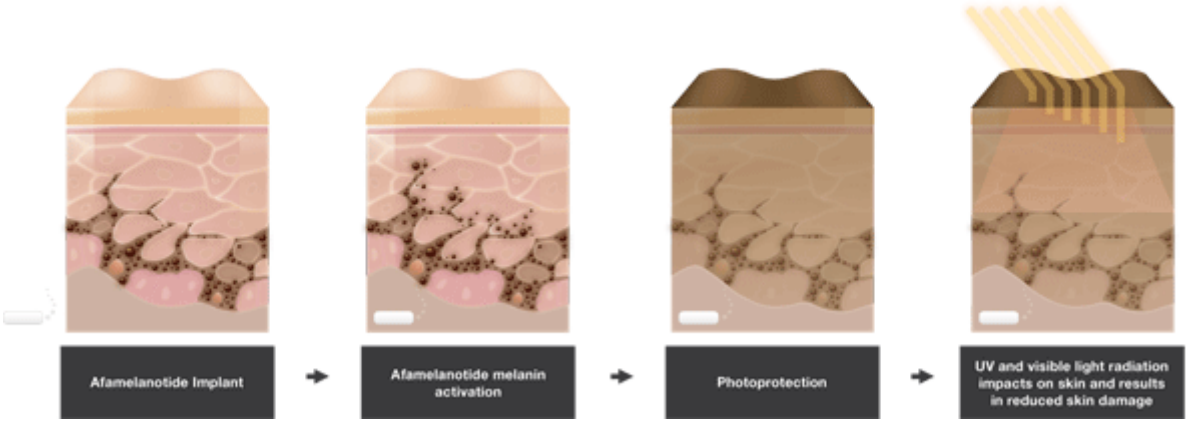
Non-ionizing radiation may further lead to lipid peroxidation in the nucleus and cytoplasm of the keratinocyte and melanocyte (the two prominent cells in the epidermis). All damaged bases described above evoke recognition steps requiring specific glycolases to remove the base, ultimately converging to common excision steps. Most prominent recovery of the genome is seen through nucleotide excision repair (NER) and base excision repair (BER), both prominent in UV induced mutagenesis.

From recent longitudinal studies, it has been demonstrated that chronically damaged skin of all patients showed an increase in constitutive melanin, correlating inversely with the severity of base pair damage. In other studies investigating one minimal erythemal dose (1 MED) of UV exposure, fair was compared to darker skin. Conclusive in all studies was that:

1. skin containing melanin suffered significantly less DNA damage in the upper and lower epidermis;
2. melanin correlated positively with rate of repair and time to repair; and
3. recovery of CPD damage in the epidermis took longer than seen for 6-4 PD.

When it comes to measuring total dermal damage following UV exposure, apoptosis (programmed cell death) provides a mechanism to prevent cells with significant DNA damage from proliferation.

Through recent findings at the National Institute of Health at Bethesda, the role of eumelanin (brown pigment) as an effective photoprotectant polymer was shown to be different from the specific role of pheomelanin. After exposure to predominantly UVB (280 to 320nm) or UVA (320-400nm) radiation, congenic mice comparing various coat colours showed that UV-irradiated melanin, particularly pheomelanin, photosensitises adjacent cells to caspase-3 independent apoptosis, and that this occurs at a frequency greater than the apoptosis induced by direct DNA absorption of UV. Pheomelanin-induced apoptosis may well be a contributive factor to the increased sensitivity of individuals with blonde and red hair to sunburn and skin cancer. In essence, a breakthrough in understanding the function of eumelanin and pheomelanin was demonstrated in recent years. Early on in life, our genetic make-up determines our constitutional quantities of the protective (eumelanin) versus the damaging pigment (pheomelanin). The latter predisposes the melanocytes to generating photoreactive pheomelanin. In later series, an in-depth review of this important finding will be published.

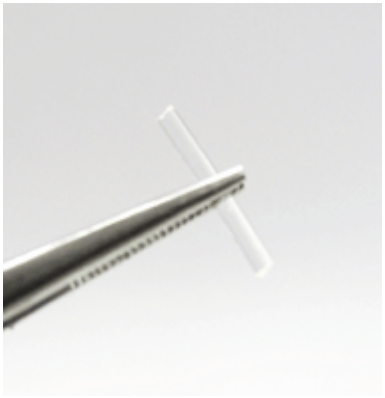


The body's response to the impact of UV is to activate natural photoprotection, melanin, in skin.

As an illustration, the pandermal damage in Caucasians (Fitzpatrick skin types I-III) is incurred within 10 minutes of UV-exposure. Clinically, the minimal erythemal dose (dilation of blood vessels expressed as redness) signifies the formation of the first photoproducts at nuclear levels. Full-thickness skin biopsies illustrate that DNA in melanocytes and keratinocytes – the two most prevailing epidermal cells – incurs damage and changes as a result of dose and intensity of radiation. This kind of dermal radiation is stochastic, as a threshold to damage is often determined. Since 1989, it has been known that the physiological release and response mechanism of α-MSH plays a role to counteract photodamage. In many ways we interpret this response as a way for human skin tissue to signal its preventative polymeric release of melanin in anticipation of the next UV rays penetrating the skin.

A breakthrough in our understanding of α-MSH came with the realisation that the majority of its secretion (99.1%) came from the epidermal cells as opposed to the -always assumed – anterior portion of the pituitary gland. Analogous to the animal system, scientists had always believed that α-MSH was centrally secreted by our cerebral tissues. Now, we have come to understand that α-MSH is actually a hormone secreted by prominent epidermal keratinocytes, as a powerful defensive mechanism against UV light. Yet, unfortunately, the majority of clinicians nowadays still believe that α-MSH is centrally secreted. The quantity of α-MSH – most often hardly detectable in the systemic circulation – lies in the region of picomolar concentrations. With this discovery, we now know that α-MSH acts as a paracrine hormone with distinct properties at ultra small concentrations and very short half-life of minutes. A further distinction was made when the US team synthetised linear and cyclic compounds. The peripheral binding of α-MSH offers opportunities to develop a medicinal therapy to prevent photodamage to the skin, and this is where afamelanotide was developed and reformulated in 2006.

At CLINUVEL, we opted to only use a linear peptide, afamelanotide, due to the fact that this is hardly able to pass the blood brain barrier (BBB). A safe and controllable mode to work with hormones is when one targets local effects, rather than centrally mediated organ effects. In afamelanotide, we have found numerous applications to use the molecule in a linear configuration. It is expected that other companies and research groups may wish to use the cyclic analogues of α-MSH in the future, but systemic control is always going to pose an issue in clinical use of cyclic analogs.
From the above it may well clarify why the physiological tanning response is a much more complex biological mechanism to shield our integument against radiation damage. Facultative use of hormones for other purposes than medicinal photoprotection is therefore not appropriate.



The 16mg afamelanotide implant

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REFERENCES

- Ichihashi M, et al, (2003). "UV-induced skin damage." *Toxicology*. Jul 15;189(1-2):21-39.
- Kadekaro AL, et al, (2005). "alpha-Melanocortin and endothelin-1 activate antiapoptotic pathways and reduce DNA damage in human melanocytes." *Cancer Res*. May 15;65(10):4292-9.
- Marrot L & Meunier JR, (2008). "Skin DNA photodamage and its biological consequences." *J Am Acad Dermatol*. May;58(5 Suppl 2):S139-48.
- Vink AA & Roza L (2001). "Biological consequences of cyclobutane pyrimidine dimers." *J Photochem Photobiol B*. Dec 31;65(2-3):101-4.