SCIENTIFIC COMMUNIQUÉ I

CLINUVEL

09 April 2018

In April and May, CLINUVEL will publish three successive SCIENTIFIC COMMUNIQUÉS to review the current understanding and progress in proopiomelanocortin (POMC) science and photomedicine. SCIENTIFIC COMMUNIQUÉ I provides an outline of the various physiological modifications taking place on proteins and the clinical relevance to our technology programs. In SCIENTIFIC COMMUNIQUÉ II we will delve into how ligands bind to the various cellular receptors, signalling cascades and output to arrive at therapeutically meaningful applications. In SCIENTIFIC COMMUNIQUÉ III we will review the effects of afamelanotide, CLINUVEL's lead drug, on the human genome. After the COMMUNIQUÉS we hope the reader will be able to grasp the, often opposing, opinions found in the abundance of literature on relevant topics.

In this series we will address some elementary aspects associated with our technology which seem to provide benefit to one patient community thus far, those with the genetic metabolic disorder erythropoietic protoporphyria (EPP).[1] In years to come the same technology may demonstrate clinical benefit in other diseases. In trying to simplify the multiplicity of our specialised scientific knowledge, we provide a background to CLINUVEL's work which assumes no, or little, scientific foundation on the part of the reader.

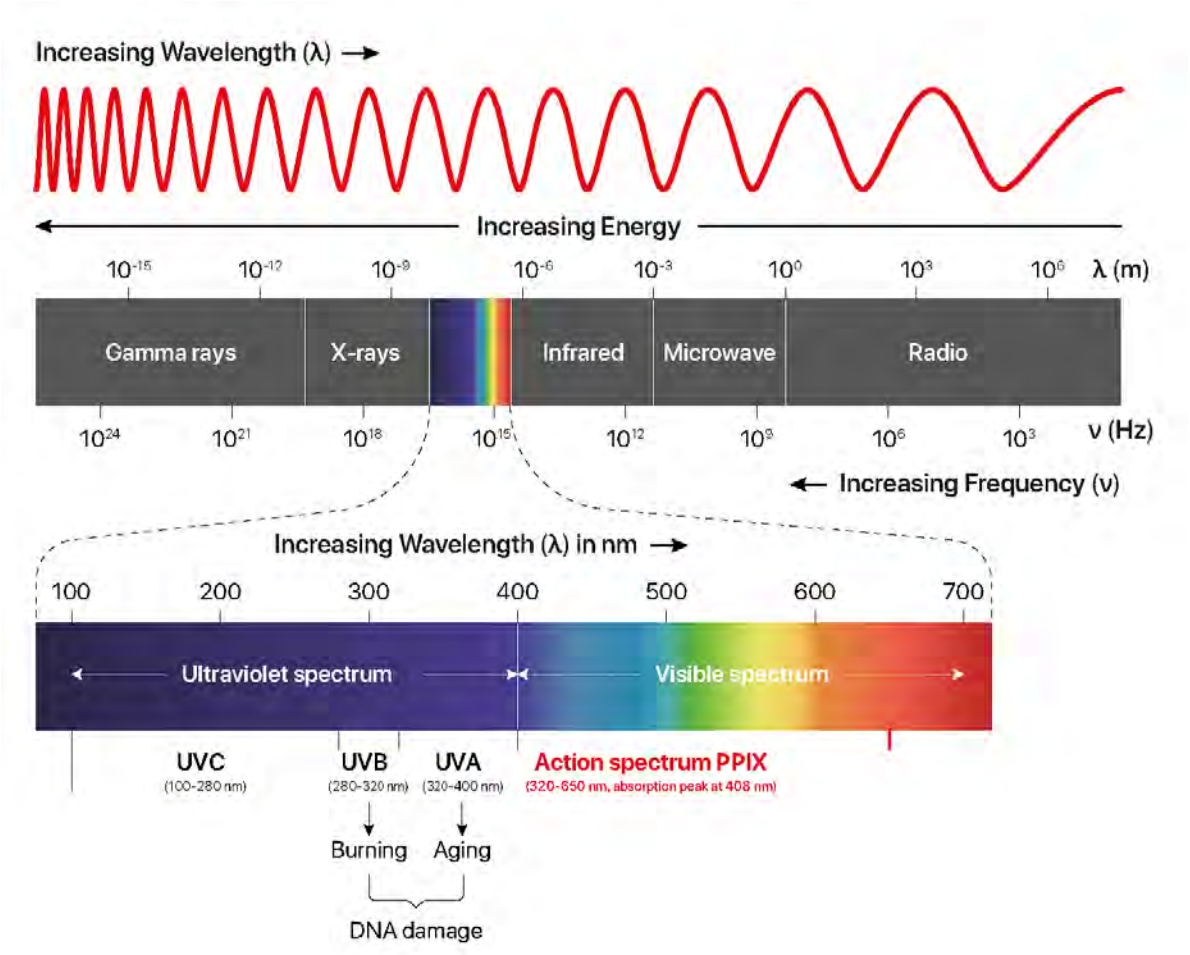

Figure 1: the electromagnetic spectrum highlighting the impacts of various wavelengths of light on skin, as well as interaction with protoporphyrin IX (PPIX).

CLINUVEL's research decisions are driven by a great number of variables; without being exhaustive, we rely on our own data as well as data generated by scientists working in related fields of interest. For this we maintain our data banks and archives, and expand our knowledge as time goes by. Across the three SCIENTIFIC COMMUNIQUÉS we will provide a schematic overview of fundamental research in POMC with the objective of sharing the expanding knowledge on our field of focus, photomedicine, with specific regard to:

(i) adult patients who lack alternative treatment; and
(ii) paediatric patient populations.

At the same time translational knowledge gained from analytical methodologies is being explored in formulation development, new therapeutic indications, and associated areas of science. The continuous expansion of value is aimed to be *concentric*. In other words, *gradual and outward progression* of clinically applicable knowledge is made as new teachings provide us with a sound scientific rationale to enter unchartered domains. In *Figure 2* the stepwise expansion of CLINUVEL's research & development is illustrated, whereby each concentric excursion away from the centre is characterised by a novel step in research.

The concentric progression from fundamental R&D (1) to the understanding of melanocyte functionality (2) is fascinating. As CLINUVEL's knowledge has increased, based on both in vivo and clinical data, so too has the scientific community expanded its understanding of melanocyte biology. This has led to fully integrative intelligence on melanocortins (3) as autocrine and paracrine mediators in light and ultraviolet (UV) induced disorders. Our loading of relevant clinical application of the knowhow led to furthering the field of photomedicine (4), and as the scientific progress advanced in oncology (5) important steps were made towards understanding protein gene expressions and signalling defects in genetically predisposed patients. CLINUVEL is moving outward (*Figure 2*) as the domains of applicable knowledge increase.

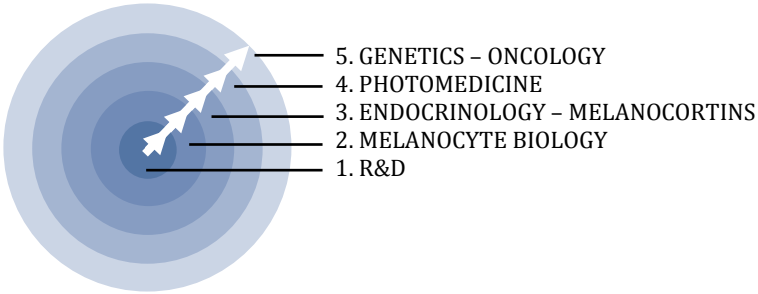

Figure 2: Concentric expansion of CLINUVEL's research & development focus.

Obviously we are not providing pro-bono data to our competitors to propel their scientific progress, and we therefore remain generic in the descriptions of CLINUVEL's scientific direction. With identified therapeutic opportunities in photomedicine, and further translation of scientific knowledge and technology in other fields of medicine, we structurally approach the various applications of POMC products.

In staying close to the present core value of CLINUVEL's pharmaceutical technology one is asked to think in terms of a vessel and its engine, ignition, and fuel required.

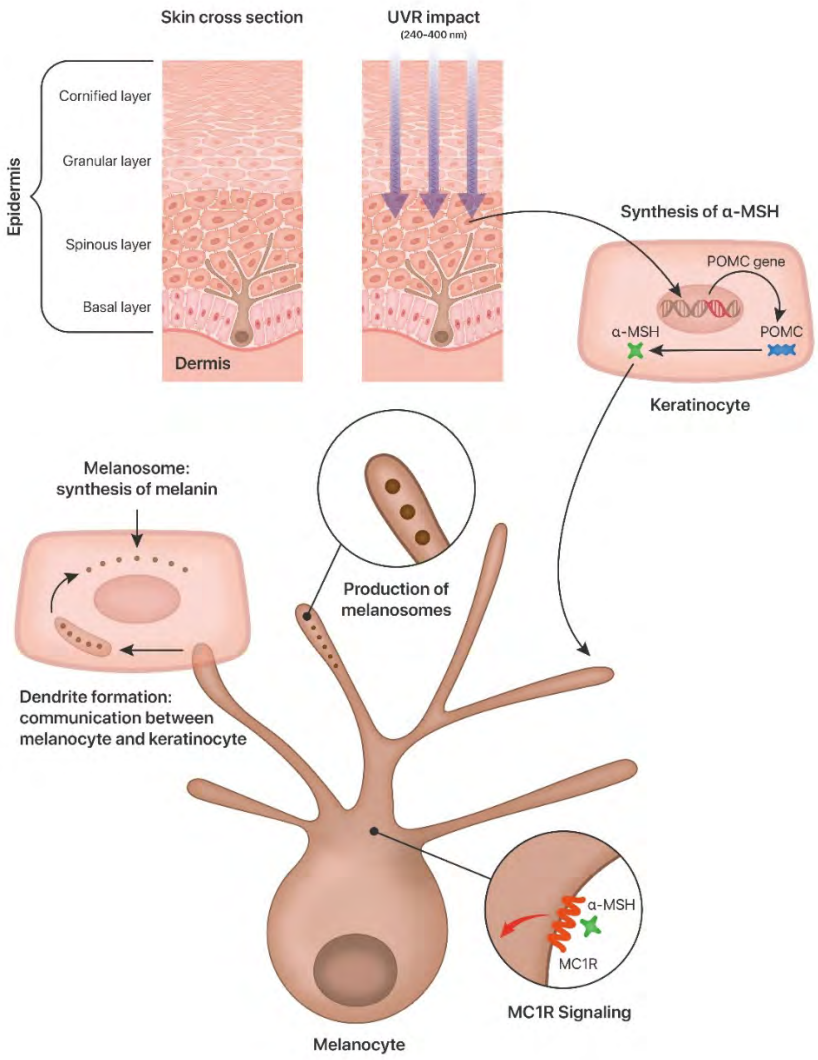

Figure 3: Two primary epidermal cells: the melanocyte and the keratinocyte.

THE ENGINE

Since we are focussing our research on afamelanotide (a synthetic analogue of alpha-melanocyte stimulating hormone, α-MSH) and several other POMCs we first start with the discussion on peptides. Naturally, therapeutic aims demand that one carefully looks at the biological activity which takes place under normal conditions within human cells. A number of intracellular processes dominate functionality of the target cells of therapeutic choice (*Figures 3 and 4*). One focus of CLINUVEL's attention is post-translational activities affecting proteins, that is after their translation by ribosomes (protein factories) is completed. For clarity, post-translational modifications are changes which occur within a cell on a protein, and generally indicate an addition of a covalent group, such as can be expected from the processes of acetylation, but other processes take place such as phosphorylation, amidation, glycosylation, neddylation, proteolysis, and protein folding to produce a functionally mature protein. One keeps in mind that most, but not all, of these post-translational processes occur to modulate the biological activities of peptides. The importance of 'changes' to proteins can also be seen, for instance, in α-MSH, which is acetylated at the N-terminus (one end of the molecule) causing paradoxical effects. Interestingly, this leads to inhibition under certain conditions, and potentiation under other circumstances. It is not uncommon in these modification processes to see dual activity affecting peptides to provide a balanced biological response and maintain homeostasis. The protein output within the cell is determined by two loci of ribosomes (organelle) within the cell.

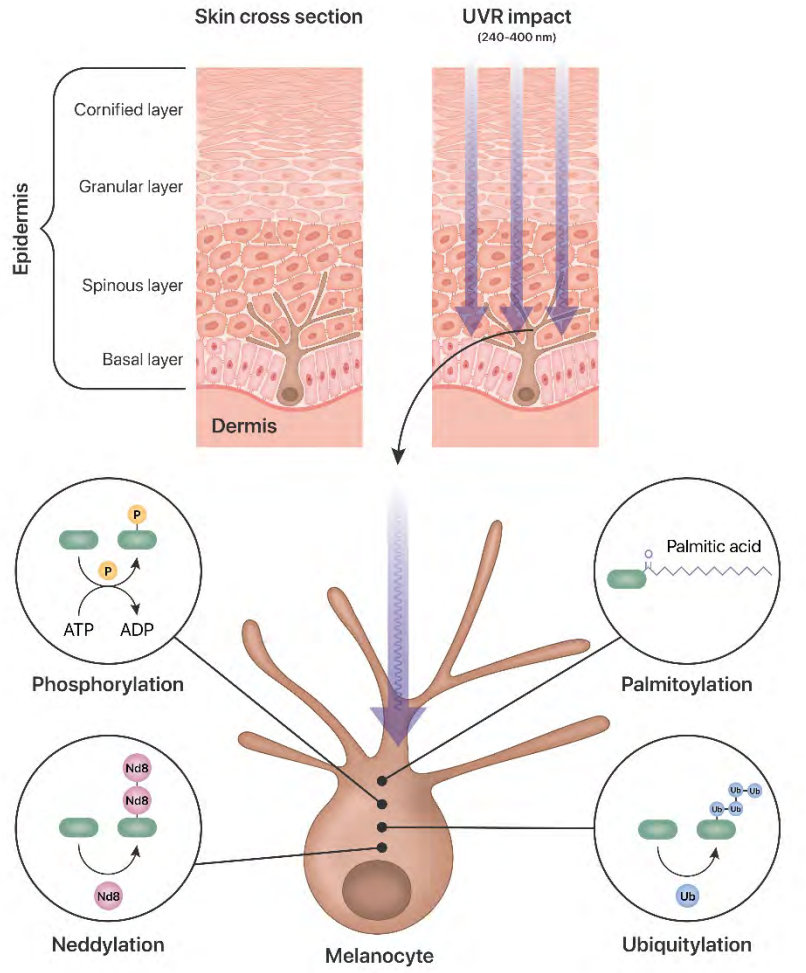

Figure 4: Protein modifications within the melanocyte.

As scientific understanding of keratinocytes and melanocytes has made significant leaps over the past decades, so too has the knowledge of the downstream communication between various proteins. With work taking place simultaneously at various laboratories and within a number of research groups, we now start to grasp the fascinating mechanisms by which various molecules are able to communicate with each other within a cell. In all the communication possibilities the master switchboard in the melanocyte is the protein microphthalmia-associated transcription factor (MITF). MITF comes in many isoforms but importantly regulates – from early human growth onwards – melanocyte development, differentiation and cell survival. The criticality of MITF is seen as various mutations in the switchboard lead to a number of genetic diseases such as frequent loss of hearing, neurological and pigmentary anomalies. We know that we rely continuously on an optimum functioning of MITF.

MITF possesses the function of transactivating various genes relating to pigmentation, differentiation, cell cycle and, importantly, apoptosis (programmed cell death). In summary the coordination of the important cellular functions of the melanocyte rests with the MITF protein. Further, MITF undergoes post-translational regulation through a number of processes such as phosphorylation, where protein kinases modify amino acid residues of the protein. A great number of modifications are taking place at the same time, but all with an aim to regulate and keep the expression of genes and proteins in check and, ultimately, to make the cellular functions act in sync at an optimum level. MITF specifically binds to so-called promotor regions of pigmentation production genes and positively regulates some key enzymes. In sync, communication also

takes place in the form of negative feedback loops as other cellular pathways try to downregulate the synthesis of pigmentation to keep the "system" in balance.

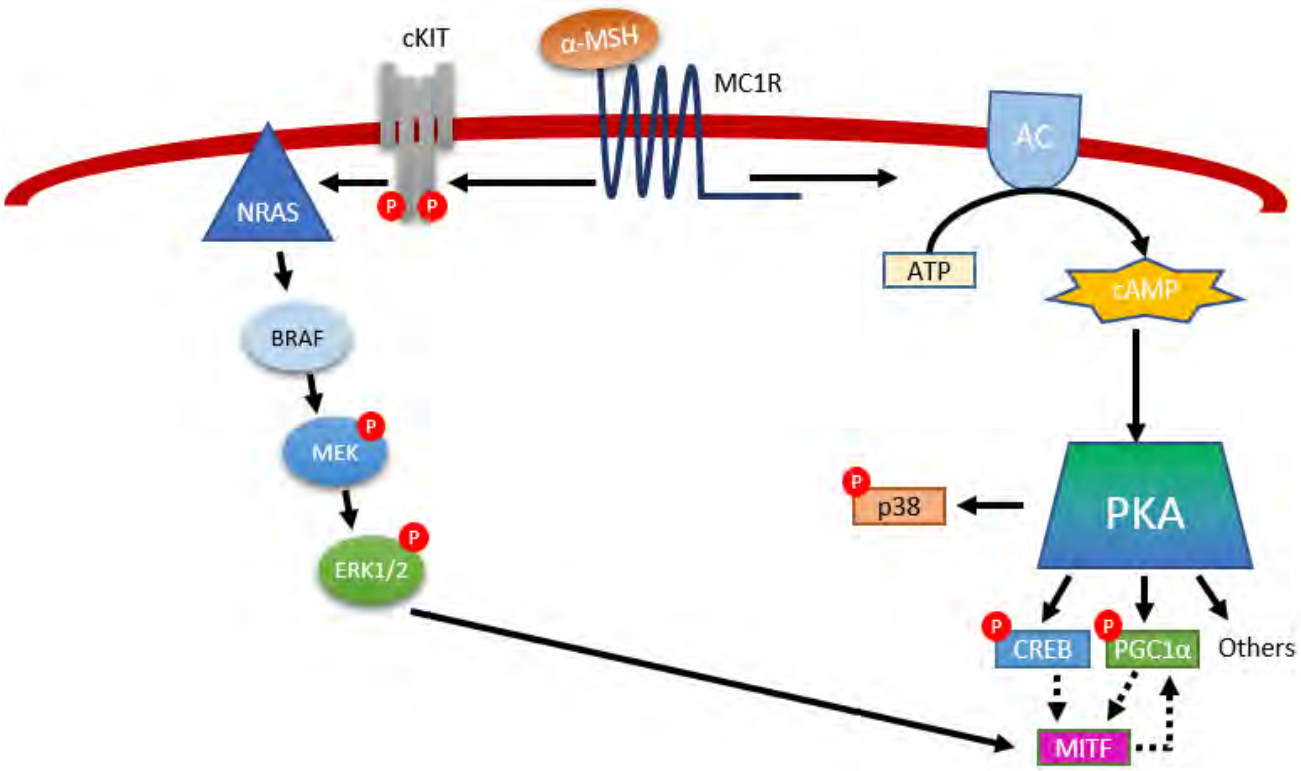

Figure 5: MC1R downstream signalling.

Other key proteins in the functioning of the melanocyte are cyclic adenosine monophosphate (cAMP) and cAMP response element binding protein (CREB). CREB is responsible for the transcriptional activity in MITF while also undergoing phosphorylation, a good example of how one protein or transcription factor communicates to another in order to optimally regulate cellular processes. CREB is essential in the skin's pigmentary response to UV radiation.

While multiple protein interaction is central to understanding the close relationship between melanocyte and keratinocyte, genetic information needs to simultaneously flow within the cells to ensure that proteins are synthesised (from DNA to RNA to protein synthesis) and that each of us can respond in the required way to stress and external stimuli, for instance light and UV radiation. One needs to keep in mind that cellular response to stimuli ultimately equals protection and restoration of balance.

A great number of transcription factors act simultaneously or sequentially, and it goes outside the realm of this overview to discuss all of these, however we don't focus our research exhaustively on gene expressions such as SOX9/10, SNAIl/SLUG, ETX, PAX3, FOXD3, BRN2, AP-1/ATF2, and LEF/TCF/β-catenin, but also on NF-κB and complementation factors (seen in chronic inflammatory diseases).

THE IGNITION

In the above we outlined some of the key elements of the identified engine room. Now we turn our attention to the trigger required to activate the melanocyte and keratinocyte before we conclude with the clinical relevance of POMC as pharmaceutical technology, specifically SCENESSE® and second generation melanocortins.

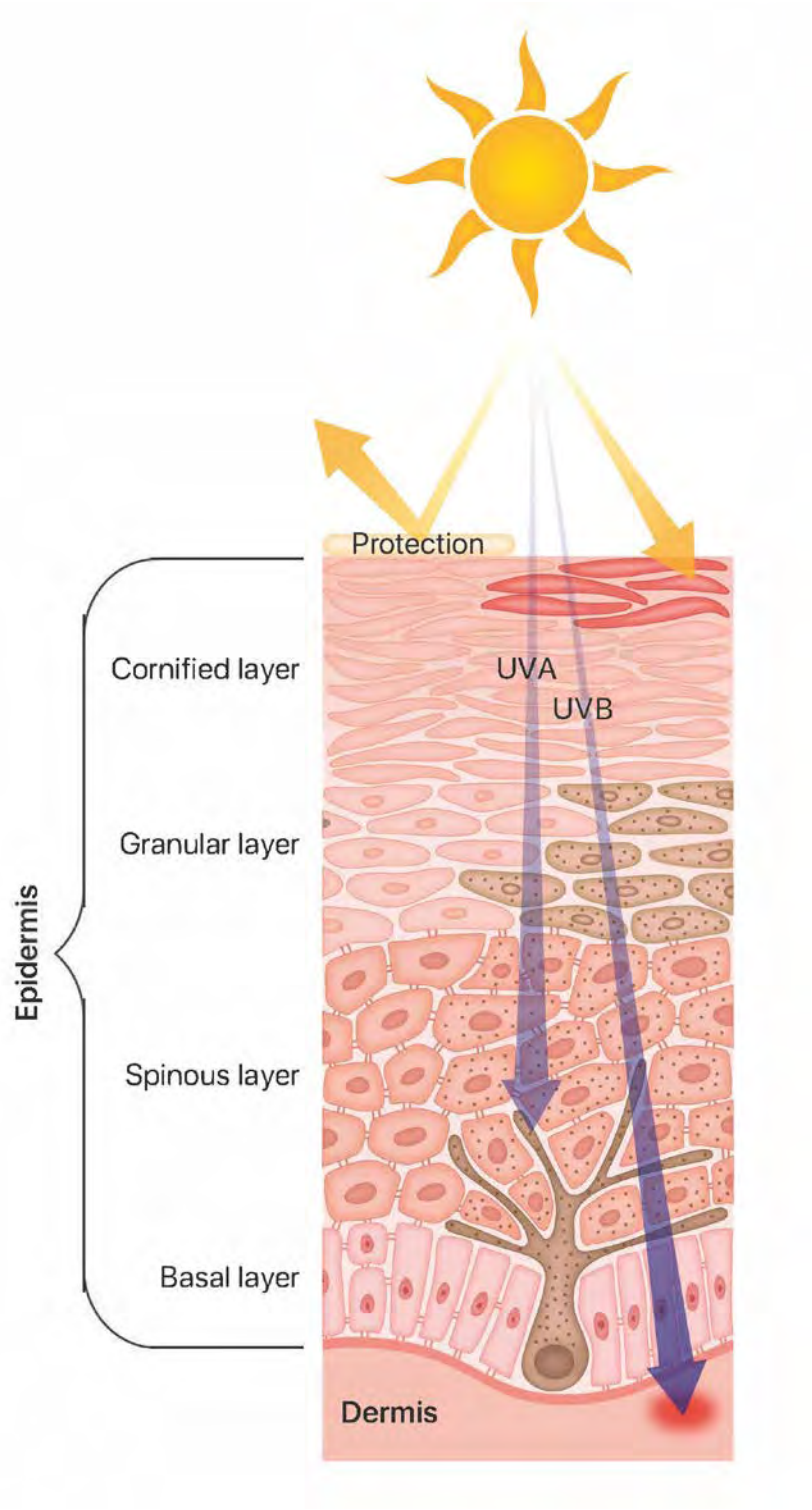

Figure 6: UV radiation impacting the epidermis.

Akin a fuel hose providing petrol to an engine, cell signalling can be ignited and initiated by various ligands and agonists. The quality of the input signal, however, determines the activation of function and therefore the quality of output required of the cell (i.e. melanocyte). Exposing skin to UV leads to the activation of a great number of proteins within keratinocytes and melanocytes. These enable a swift response to cellular stress signals and allow the cells to prelude the damage which takes place in the various cellular organelles and nucleus. Two of the peptides expressed under physiological conditions are α-MSH and desacetyl-α-MSH.

However, both of these hormones are released by humans in minute quantities (picomolar concentrations) and appear quite ineffective at protecting Caucasian skin types (Fitzpatrick I-III) under 'stress' or attack.

We now understand how UV irradiation of skin elicits a number of immediate and prolonged reactions, and we first zoom in on the instantaneous reactions taking place within skin cells. UV exposure activates the p53 gene, among many others. Like other proteins, p53 is phosphorylated to ensure activity and stabilisation within the cell. In brief, p53 is a ubiquitous human tumour suppressor protein controlling the cellular response to DNA damage, cycle progression and apoptosis by regulating its targets transcriptionally. An intact p53 gene is essential for an adequate anti-tumour response; in many cancers loss-of-function mutations and deletions in the gene are frequently observed. p53 also plays a critical role in the normal UV stress response and activation of pigmentation by transcriptional activation of the POMC gene.

Figure 7: The fuel provided determines the output of the target cell.

Sun exposure simultaneously leads to activation of stress mitogen activated protein kinases (MAPKs) and p38, a delta protein kinase. Under normal conditions the p38 protein kinase controls cell differentiation. Under stress, however, it regulates a *cellular distress response*. The p38 gene "talks" to a transcription factor USF-1 which regulates the melanocortin-1 receptor (MC1R; the docking station for afamelanotide, see below *The Fuel*) and the expression of the hormone α-MSH. This is yet another example of how UV evokes a number of cellular reactions which work *independently* to ultimately protect the human body from damage.

Through another pathway, prostaglandin E2 (PGE2; a lipid signalling intermediate), a number of cellular reactions can be invoked which resemble the UV response described above. It has even been reported that PGE2 can provoke, in certain circumstances when administered to the melanocyte, irregular eumelanin output and therefore visible darkening of the epidermis. This is due to the fact that human melanocytes express two PGE2 receptors which, when stimulated, can increase cAMP and key enzymes within the cell.

The list of potential proteins playing a role or eliciting a melanogenic response within the melanocyte is far from complete, but we've represented an overview of a number of factors which are relevant when viewing the response of skin to light and UV.

THE FUEL

For the purpose of the SCIENTIFIC COMMUNIQUÉS, we have restricted the focus on communication (signalling) pathways of the melanocyte – protein communication channels – to one receptor, the MC1R, surrounded by other four different cell receptors: endothelin-1, stem cell factor, fibroblast growth factor, and frizzled. All of the known communication pathways running south are coming together in the switchboard MITF (see above, *The Engine*).

The ultimate objective is to generate the "right" kind of pigmentation (eumelanin) and prevent the formation of the photoreactive pigmentation (pheomelanin) which dominates in fair skinned individuals. Eumelanin in skin is shown to effectively absorb light and UV. This brown pigment is known to be more effective in energy dissipation than the reddish-yellow pheomelanin.

The pigment pheomelanin, however, reacts strongly with UVA (wavelength 320-400nm) and is shown to *aggravate* skin damage from UV exposure. Thus, not only are red haired individuals genetically 'given' the inferior pigment, they also carry an unfavourable ratio of a substance which reacts with UV under all conditions. In SCIENTIFIC COMMUNIQUÉ II we will discuss the pigmentary output and various effects it has as a response to photodamage.

UV exposure leads to oxidative stress and formation of photoproducts in cells, which needs to be avoided long-term. Activating a pathway early and efficiently through one of the receptors mentioned above can significantly reduce the damage to the keratinocyte and melanocyte. Along this reasoning, it has been shown that pharmacological intervention, administering melanocortins to initiate a "positive" signal, makes much sense biochemically and clinically.

RELEVANCE TO CLINUVEL'S SCIENTIFIC PROGRAMS

The skin is a most complex and sizeable organ of the human body. Dermatologists colloquially call the skin the "hormone factory". Various proteins, receptors and hormones are expressed by the skin, and some of these belong to the POMC family, including α-MSH. CLINUVEL has developed the synthetic analogue afamelanotide as part of the POMC family to simulate and mimic the required physiological response following stress signals. In simpler terms, thus far afamelanotide has proven to be clinically the most effective POMC hormone to protect against light and UV damage.

The quest to arrive at *an optimum systemic photoprotective strategy* has been successfully demonstrated by injecting afamelanotide for the prevention of symptoms in a number of "light induced diseases", or photoinduced disorders, and research is continuing under CLINUVEL's guidance. Systemic use of POMC is regarded by photophysicists and photodermatologists as a therapeutic breakthrough.

In order to understand how POMC hormones and afamelanotide exert their activity within the entire surface of the human epi-/dermis, we provided here the key elements of cellular protein communication. Deeper understanding also provides some insight in the directional decisions CLINUVEL has taken to develop analogues for preventative and curative purposes in a range of diseases within the domain of photomedicine.

As previously published, the notion of "tanning" is, in reality, a stress response to light and UV damage. This damage is incurred in some parts of the epidermis in an attempt to elicit a signal to protect cells located deeper within the dermis. The "tanning" of the top layer of the skin is divided into a number of stages dependent on the length of exposure. The ability to efficiently respond to UV stress is determined by one's constitutive ratio of eumelanin versus pheomelanin production; we will further elaborate on this in SCIENTIFIC COMMUNIQUÉ II.

Learnings from the past 14 years refer to the efficiency of **provoking a (photo)protective response** in patients who are at extreme risk from light and UV. The use of a novel configured drug *under extreme*

conditions in ultra-rare patient populations teaches us much which could be applicable to other analogues, and in other populations.

Away from the domain of light and UV protection, for decades the scientific focus globally has been to unravel the steps in melanoma formation. Naturally, since a prominent skin malignancy – melanoma – originates from a melanocyte, our teams also concentrated their efforts on understanding these mechanisms. Only in 2002 was it confirmed by researchers that melanoma (a wide term used for what is now understood to be several disease entities) is principally a tumour originating from underlying genetic defects. By contrast, tumours such as epidermal squamous cell carcinomas are mainly due to chronic sun damage and whereby the genetic component is not as prominent as in melanoma. Since the melanocyte gives rise to the genesis of melanoma it is logical that our teams have spent abundant manhours over more than a decade to expand and update their knowledge on this subject.

The core expertise of CLINUVEL's scientific teams resides in photomedicine, the interaction of light and human biology and matter (photophysics). Whereas we have concentrated our research efforts on a number of indications which are affected by light, the R&D work will continue to expand outward to closely related fields of medicine and pharmacology.

In summary, the detailing of the *Engine, Ignition, and Fuel* above is aimed to explain in simpler terms how CLINUVEL's technology, afamelanotide and further peptides under development, is influencing and is being affected by various other cellular proteins and processes.

Optimising molecular processes enables adequate response to light and UV insult to skin, and this is seen – for instance – by the use of afamelanotide in EPP patients, who are intolerant to light emitted along the visible spectrum.

TAKE AWAYS - SCIENTIFIC COMMUNIQUÉ I

- concentric expansion of CLINUVEL's R&D focus
- within the melanocyte and keratinocyte proteins communicate with each other
- communication pathways aim to protect cells from damage
- afamelanotide provides adequate downstream signals to the melanocyte and keratinocyte
- pigmentary (eumelanin) output is a protective response to light and UV induced stress and attacks
- CLINUVEL focuses its research on photomedicine, a number of disorders induced by light and UV radiation

[1] SCENESSE® (afamelanotide 16mg) is approved in Europe as an orphan medicinal product for the prevention of phototoxicity in adult patients with EPP. Information on the product can be found on CLINUVEL's website at www.clinuvel.com.