Xeroderma pigmentosum (XP)

CLINUVEL

In this technical note, six brief chapters are discussed

i. DNA repair process in healthy individuals
ii. Disturbed DNA repair in XP patients
iii. Afamelanotide and assisted DNA repair
iv. Relevance of CUV156 (XPC) first results
v. Executive summary
vi. Epilogue background XP
vii. References

Note to readers: *the executive summary provides a simplified explanation, including modes of action and relevance of the first results from the use of afamelanotide in XPC patients (the CUV156 study).*

i. DNA skin repair in healthy individuals

Generally, DNA damage caused by ultraviolet (UV) light is efficiently repaired by skin cells through basal excision repair (BER) and/or nucleotide excision repair (NER). As one grows older and cellular damage accumulates, repair mechanisms lose efficiency and the risk of incurring photodamage and carcinogenesis increases, depending on one's skin complexion, genetic make-up and immune response.

The focus here is on NER of single-strand DNA damage (SSB) caused by UV radiation. NER consists of two components: global genomic repair (GGR); and transcription coupled repair (TCR). From **diagram 1**, one sees a summary of the NER pathways, whereby the emphasis lies on the GGR when it comes to single-strand DNA repair processes in skin cells. Whereas oxidative lesions such as 8-oxo-dG are usually effectively removed through the BER pathways, larger photoproducts such as PPs and cyclobutane pyrimidine dimers (CPDs) are repaired via the NER. Uncompromised

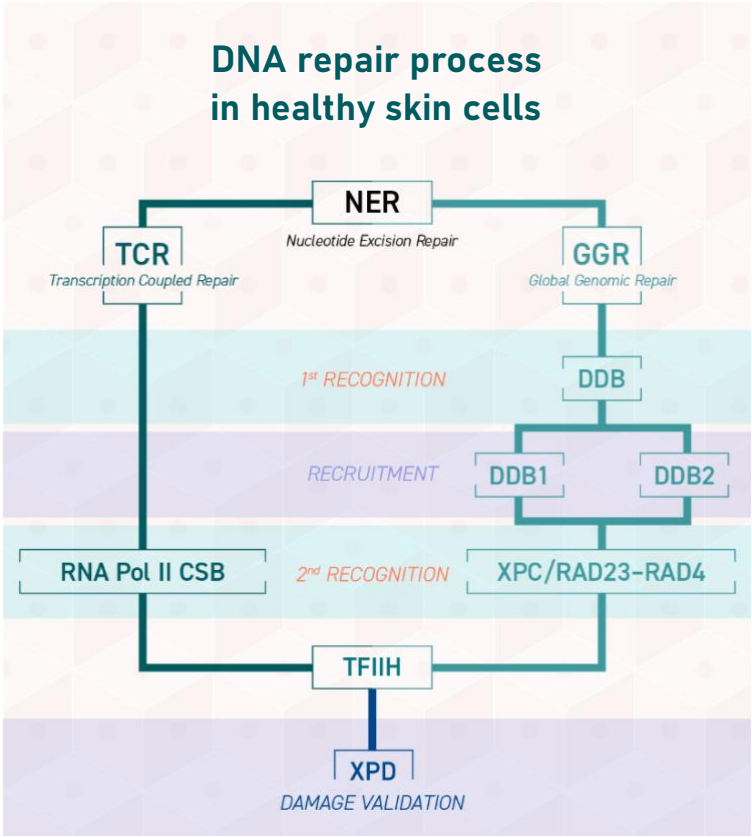

Diagram 1 *The NER pathways and main genes expressed*

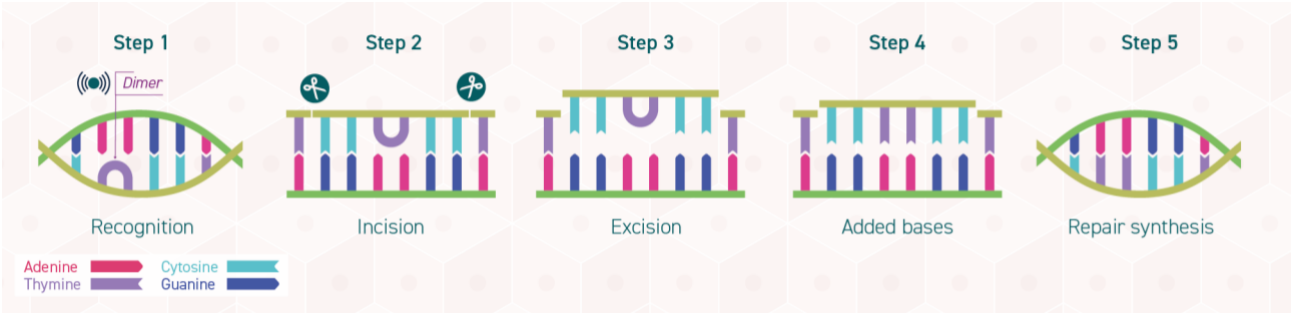

Diagram 2 *The NER process*

DNA skin repair in healthy individuals follows five steps **diagram 2**:

1. Recognition
2. Incision
3. Excision
4. Added bases
5. Repair synthesis

ii. Disturbed DNA repair in XP patients

In XP patients, depending on the genetic defect concerning a specific complementation factor (seven complementation groups and one variant), UV damage affects specific parts of the DNA regenerative process.

7 complementation factors in DNA repair processes	
XPA	sensing DNA damage and assisting in unwinding the helix
XPB	open complex formation in strand repair
XPC	sensing DNA damage
XPD	open complex with TFIIH
XPE	assist unscheduled DNA synthesis, recruitment
XPF	involved in excision during NER
XPG	incise the damaged strand respectively 5′ and 3′ to the lesion

Table 1 *Functions of complementation factors*

XPA patients present with both skin and neurologic manifestations of the disease.

The **XPB mutation** is associated with Cockayne syndrome.

XPC patients are most intolerant to light (HEV) and UV, and develop frequent skin cancers eventually leading to a high mortality. When mutated, the endonuclease is unable to sense damage in DNA, resulting in defective genomic regeneration.

XPD mutations can result in XPD, Cockayne syndrome or trichothiodystrophy (TTD).

XPE patients have a high incidence of skin cancers which develop late in their life.

XPF patients suffer a relatively mild disease, with a later onset of skin cancer, and some may develop neurologic impairment or growth defects, and are then classified as having Cockayne syndrome.

XPG patients may suffer sun sensitivity, skin cancers and those with truncation mutations have combined features of XP and Cockayne syndrome.

XPV patients have a high incidence of skin cancers.

All seven complementation factors and the pol eta play a role in the DNA regeneration following genomic insult.

iii. Afamelanotide and assisted DNA repair

Without being complete, a summary of the repair processes is described. CLINUVEL focuses on the clinical evaluation of afamelanotide in patients diagnosed with either XPC or XPV.

In various studies, **afamelanotide and the naturally occurring hormone α-MSH** have been shown to positively affect and assist the NER (and BER) processes following UV radiation. A summary of the seven most prominent activities is provided, afamelanotide:

1. **optimises cellular signalling** via melanocortin-1 receptor (MC1R), stimulating a natural, physiological process.
2. induces phosphorylation of the ATR-ATM genes via PKA leading to mediation of DDB and **recruitment of XPC** to the DNA damage sites.
3. induces NR4A2 to DNA damage via p38 pathway **recruiting XPC**.
4. induces the ATR-ATM pathway, thereby **promoting γH2AX**, a DNA damage sensor.
5. **enforces the p53 response**, thereby targeting p21 and GADD45 to assist single strand damage-repair via BER.
6. **enhances the BER** by upregulating OGG1 and APE1.
7. reduces oxidation of purine and pyrimidine bases.

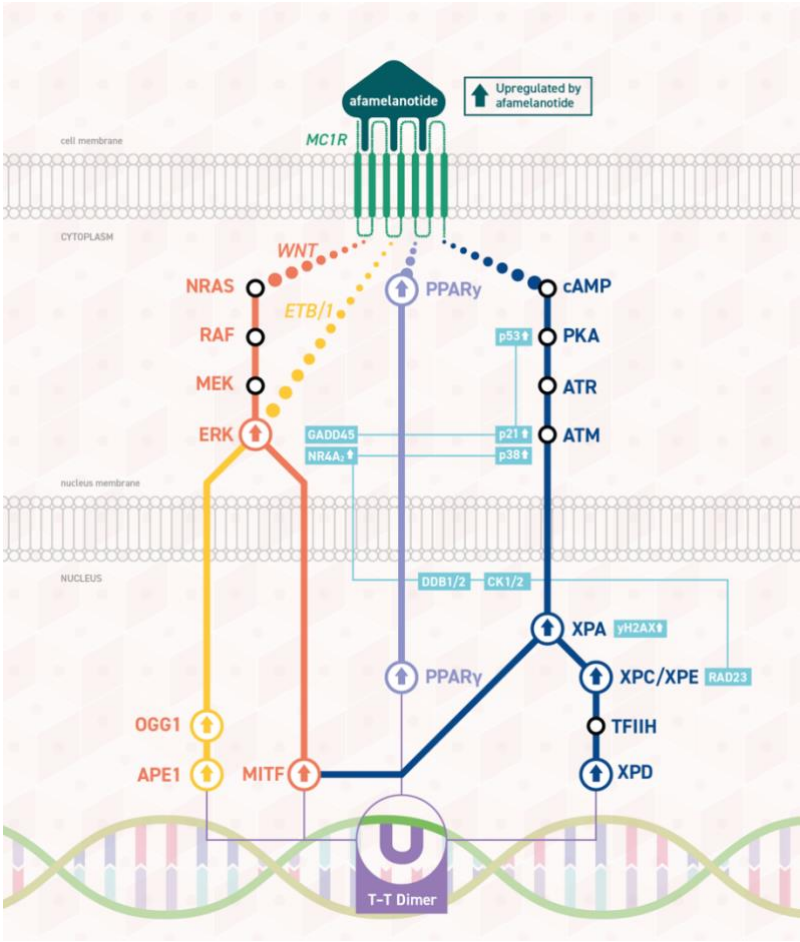

Diagram 3 *Afamelanotide positively influencing cellular pathways assisting DNA repair via NER and BER*

Afamelanotide has been shown in earlier experiments to clear oxidative photodamage (ROS) causing strand-breaks and 8-oxo-dG. In other research studies, natural α-MSH promoted the translocation of PPARγ to the nucleus of the cell, thereby promoting repair and photoprotective mechanisms.

Important is the realisation that *afamelanotide acts on several cellular pathways simultaneously*, as illustrated in **diagram 3**. In general, only the biological ligand and its analogues will exert this effect, other MC1R agonists do not fully or efficiently activate these pathways, as shown with forskolin and cAMP modulators and new molecules being tested.

In summary, various experiments have shown that afamelanotide protects DNA integrity. Today's first clinical results from CUV156 - evaluating afamelanotide in XPC patients - seem to confirm earlier findings.

iv. Relevance of CUV156 (XPC) first results

In the three XPC patients evaluated, consequent to UV irradiation of exposed skin, skin biopsy analyses have shown that afamelanotide administration on days 0, 14, 28, 42, 56 and 70 resulted in a decrease of photoproducts, expressed as a reduction on CPDs (see **table 2**).

The decrease in photodamage shown is clinically meaningful in that DNA skin damage has been reduced, thereby lowering the principal risk factor of skin cancer (chromatin replication by cell division) development, in a patient population known to be extremely sensitive and intolerant to UV light.

Whereas various research groups and publications have shown the efficiency of afamelanotide in cellular studies and animal studies, CUV156 *is the first trial globally in which a systemic melanocortin has been used in XP* (**see references 1-15**).

Interim results CUV156 (XPC, n=3)					
	CPDs	**p53**	**γH2AX**	**MED**	**MD**
Patient 1	-48.0%	-15.2%	+17.1%	decrease	increase
Patient 2	-17.1%	+36%	+1.65%	increase	increase
Patient 3	-4.5%	+16.5%	+15.47%	increase	increase
Overall clinical assessment	Positive results	Variable results, increase	Positive results	Variable results, positive	Positive results

Table 2 *First results of CUV156, evaluating afamelanotide in three XPC patients*

The use of afamelanotide as a systemic photoprotective drug has been shown in erythropoietic protoporphyria (EPP), and these first results in XPC indicate a further use of the drug substance. In general, XP patients are characterised as having **the highest need of systemic (body-over) photoprotection** given their lifelong *extreme risk of incurring skin cancers and high mortality*.

The decrease in CPDs indicates the reduction of photoproducts induced by UV irradiation, while the increase in p53 and γH2AX suggests cellular optimisation and genomic protection by afamelanotide. The increase in minimal erythemal dose (MED), decrease of erythema at the highest UVB dose given to the patient and increase in melanin density (MD) points to a barrier protection offered by the therapy.

Relevance of CUV156 results in XPC

- increasing tolerance to UV without erythema ('sunburn')
- optimising cellular functions (signalling)
- increasing natural (biological) defence to UV
- decreasing photodamage (photoproducts)
- eliminating oxidative damage to skin (ROS)
- increasing melanin in skin (mechanical protective barrier and antioxidative action)

Table 3 *Benefits of results of CUV156 for wider populations at risk of solar damage.*

Table 3 lists the main benefits derived from the observations made in study CUV156, and extrapolated to wider populations at risk of UV and HEV radiation, known as polychromatic exposure. Since UVA and UVB are known carcinogens, the prevention of photodamage is of high relevance to individuals prone to photodamage.

Clinuvel therefore focuses in parallel to the XP population on three categories:

1. family/personal history of skin cancers
2. immune suppressed, and
3. extreme outdoors.

v. Executive summary

In XP patients, there is *the highest known need* for systemic therapy to reduce, abate or annihilate photodamage caused by UV and HEV light sources.

During past years, a number of studies and experiments have shown how the biological ligand to MC1R, α-MSH – and cross-talking within other cellular pathways – could assist in the repair of UV-provoked DNA skin damage. For the first time, the melanocortin afamelanotide has shown to assist the repair of DNA damage in XPC patients, known to be at the highest risk of UV and to develop frequent skin cancers leading to a short life expectancy. Afamelanotide had already been shown to be a potent systemic photoprotective agent against UVA, UVB and HEV in the severe light intolerance disorder EPP.

In the three XPC patients evaluated, a reduction of 4.5%, 17.1%, and 48% in CPDs (photoproducts, DNA damage) was found. Given that one is exposed to polychromatic light consisting of 5.1% UVA, 0.3% UVB and

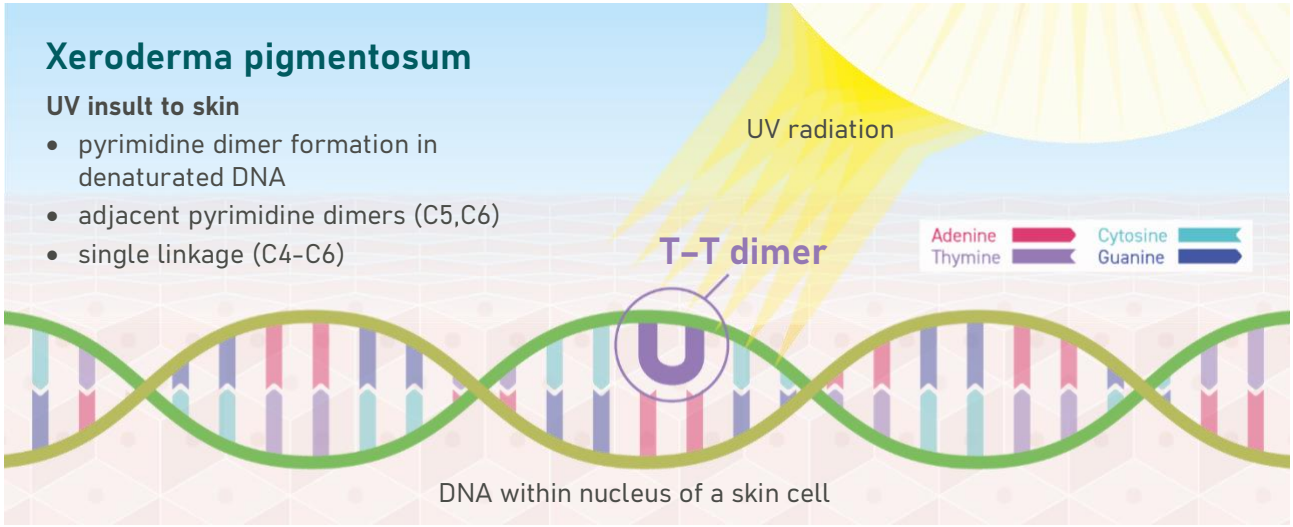

Diagram 4 *Most frequent UV damage in the form of pyrimidine dimers in XP*

62.7% HEV, the ability to reduce photoproducts caused by mutagenic UVB is a strong indication that the drug under investigation is able to reduce and modulate the absolute risk factor of skin cancer(s).

From the findings of CUV156, it is seen that combination of analyses strongly indicates that afamelanotide offers genomic protection by assisted DNA repair and systemic photoprotection.

After years of preparation and discussions with Ethics Committees, National Competent Authorities and the medical expert community, CLINUVEL's teams exceptionally obtained the permission to test afamelanotide in XPC patients. Historically, XP patients have been protected from participating in clinical trials given their high morbidity, tumour burden and short life expectancy. We are grateful for the consent and participation of patients and families, and sincerely hope we are contributing to a medical solution for the XP community worldwide.

The relevance of today's first results lies in afamelanotide's ability to

i. **reduce photoproducts in a highly susceptible population,**
ii. **positively influence skin cells, causing increase in γH2AX and p53 (variable), and**
iii. **increase the tolerance to UV, observed by increase in MED and decrease in erythema ('burn').**

The opportunity to provide genomic stability following UV insult to the skin is clinically and commercially significant. Having tested the drug in the most extreme and UV-intolerant population, it is conceivable that afamelanotide and other melanocortins are of use in larger populations at risk of solar (photo-)damage and skin cancer.

CLINUVEL is moving forward with its studies CUV156, CUV152 and CUV154 in XPC and XPV patients. Results will be shared when analyses of data have been received from the medical centres.

vi. Epilogue background xeroderma pigmentosum (XP)

XP is a recessive genetic disorder, whereby patients have an extreme 'sensitivity' to UV light, a 1,000-fold increase in skin cancers from age two onwards and a life expectancy of approximately 30 years. XP has a worldwide population frequency of approximately 1 in 250,000 but has a higher frequency in Japan and around the Mediterranean. There are seven genetic subgroups of XP, which are all resultant of pathogenic mutations in genes within the nucleotide excision repair (NER) pathway, as well as a XP variant resultant of a mutation in translesion synthesis (TLS), POLH.

Following light and UV exposure, UV-light induced DNA damage both distorts and covalently modifies DNA (nucleus of the cell), requiring a precise NER process to undo the damage incurred. In absence of functional NER mechanisms XP patients suffer up to 2,000 times greater susceptibility to uniformly distributed melanomas and 10,000-fold increase in basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) skin cancers. Often the frequent occurrence of these cancers leads to a compromised and short life. XP is a life-threatening disorder,

whereby patients develop multiple skin cancers each year, and which require mutilating surgeries; patients spend lifelong shielded from light sources and UV

UV and high-energy visible (HEV) radiation cause oxidative damage through the formation of radical oxygen species (ROS) formed in exposed skin surface. Secondary consequences of ROS are known as lipid peroxidation, protein oxidation and DNA oxidation particularly of guanosine nucleotides such as the formation of 8-oxo-deoxyguanosine (8-oxo-dG). Direct UV-induced DNA skin damage appears as photoproducts, consisting of those listed in **table 4**.

Photodamage = photoproducts in skin	
a	pyrimidine dimers (PD)
b	cyclobutane pyrimidine dimers (CPDs)
c	Dewar isomers
d	pyrimidine hydrate
e	thymine glycols
f	dipurine adducts

Table 4 *The variety of photoproducts following UV exposure*

Complementation factors in XP

The **XPA gene** codes for DNA damage binding protein 1 (DDB1), found on chromosome 9q22, needed for sensing DNA damage and assisting in the unwinding of the helix. The **XPB gene** codes for excision-repair cross-complementing 3 (ERCC3) found on chromosome 2q21; the ERCC3 is a helicase and the largest part of a 9-subunit protein complex (TFIIH) needed for open complex formation in DNA repair. **XPC** is characterised by a defect on chromosome 3p25, and codes for an endonuclease needed to sense damage from severe sun burn and malignant tumor formation of the skin and mucous membranes. The **XPD gene** codes for ERCC2 and is found on chromosome 19q13, needed to form an open complex with TFIIH directing repair to start gene transcription. The **XPE gene** codes for DDB2 and is required for recognition of UV-damaged DNA together with DDB1 and allows recruitment of other proteins needed for NER and is found in the chromosome 11p11.The **XPF gene** is located on chromosome 16p13 and codes for ERCC4, which usually forms an endonuclease with ERCC1 that incises damaged DNA. The **XPG gene** codes for ERCC5 and is on chromosome 13q33, required for the incision needed during NER and cofactor to remove pyrimidines from DNA. The **XPV gene** codes for polymerase eta found on chromosome 6p21, not considered part of NER but is involved in replication of past DNA lesions.

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CLINUVEL has issued a separate general release on the first results from the CUV156 study. Please go to www.clinuvel.com.

About CLINUVEL PHARMACEUTICALS LIMITED

CLINUVEL (ASX: CUV; ADR LEVEL 1: CLVLY; XETRA-DAX: UR9) is a global specialty pharmaceutical group focused on developing and commercialising treatments for patients with genetic, metabolic, systemic, and life-threatening, acute disorders, as well as healthcare solutions for specialized populations. As pioneers in photomedicine and the family of melanocortin peptides, CLINUVEL's research and development has led to innovative treatments for patient populations with a clinical need for systemic photoprotection, assisted DNA repair, repigmentation and acute or life-threatening conditions who lack alternatives.

CLINUVEL's lead therapy, SCENESSE® (afamelanotide 16mg), is approved for commercial distribution in Europe, the USA, Israel, and Australia as the world's first systemic photoprotective drug for the prevention of phototoxicity (anaphylactoid reactions and burns) in adult patients with erythropoietic protoporphyria (EPP). Headquartered in Melbourne, Australia, CLINUVEL has operations in Europe, Singapore, and the USA. For more information, please go to https://www.clinuvel.com.

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innovative R&D efforts; the effectiveness of our patents and other protections for innovative products, particularly in view of national and regional variations in patent laws; our potential exposure to product liability claims to the extent not covered by insurance; increased government scrutiny in either Australia, the U.S., Europe, China and Japan of our agreements with third parties and suppliers; our exposure to currency fluctuations and restrictions as well as credit risks; the effects of reforms in healthcare regulation and pharmaceutical pricing and reimbursement; that the Company may incur unexpected delays in the outsourced manufacturing of SCENESSE® which may lead to it being unable to supply its commercial markets and/or clinical trial programs; any failures to comply with any government payment system (i.e. Medicare) reporting and payment obligations; uncertainties surrounding the legislative and regulatory pathways for the registration and approval of biotechnology based products; decisions by regulatory authorities regarding approval of our products as well as their decisions regarding label claims; any failure to retain or attract key personnel and managerial talent; the impact of broader change within the pharmaceutical industry and related industries; potential changes to tax liabilities or legislation; environmental risks; and other factors that have been discussed in our 2022 Annual Report. Forward-looking statements speak only as of the date on which they are made, and the Company undertakes no obligation, outside of those required under applicable laws or relevant listing rules of the Australian Securities Exchange, to update or revise any forward-looking statement, whether as a result of new information, future events or otherwise. More information on the forecasts and estimates is available on request. Past performance is not an indicator of future performance.

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