



REVOLUTIONARY UVB PROTECTION

AhR Antagonist - Benzylidene
Dimethoxydimethylindanone

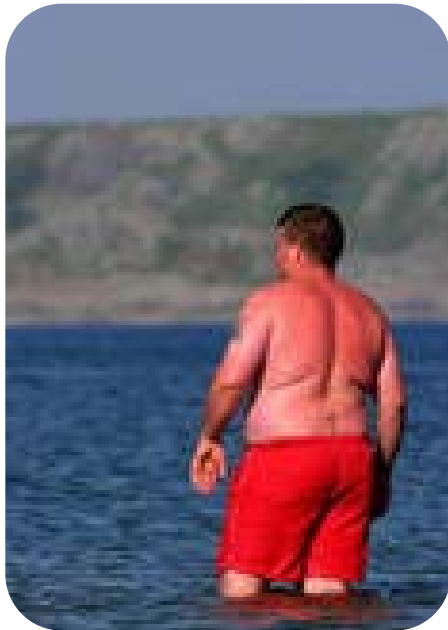
SUN CARE & BEYOND

February 2010
IMWSCC

UVB DAMAGE

ESTABLISHED THINKING

- **Sunburn (Erythema) – Release of inflammatory agents**
 - **Warning – Get out of the sun!**



UVB DAMAGE

ESTABLISHED THINKING

➤ DNA Damage

➤ (P53 gene, dimerization of thymine bases)

➤ Non-Melanoma Skin Cancer

➤ Malignant Melanoma



Basal cell carcinoma



Squamous cell carcinoma

UVB DAMAGE

ESTABLISHED THINKING

- **Photo Aging (Premature Aging) – Release of matrix metallo proteases (MMPs)**



UVB DAMAGE

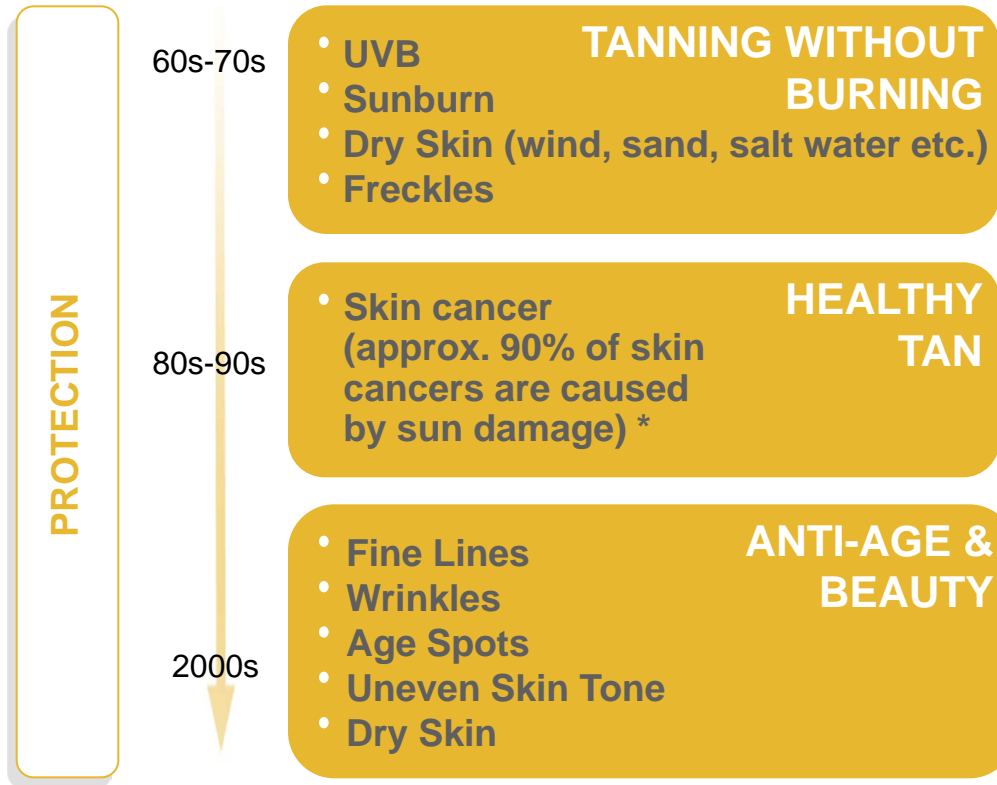
ESTABLISHED THINKING

- **Immune Suppression – Destruction of Langerhans cells**



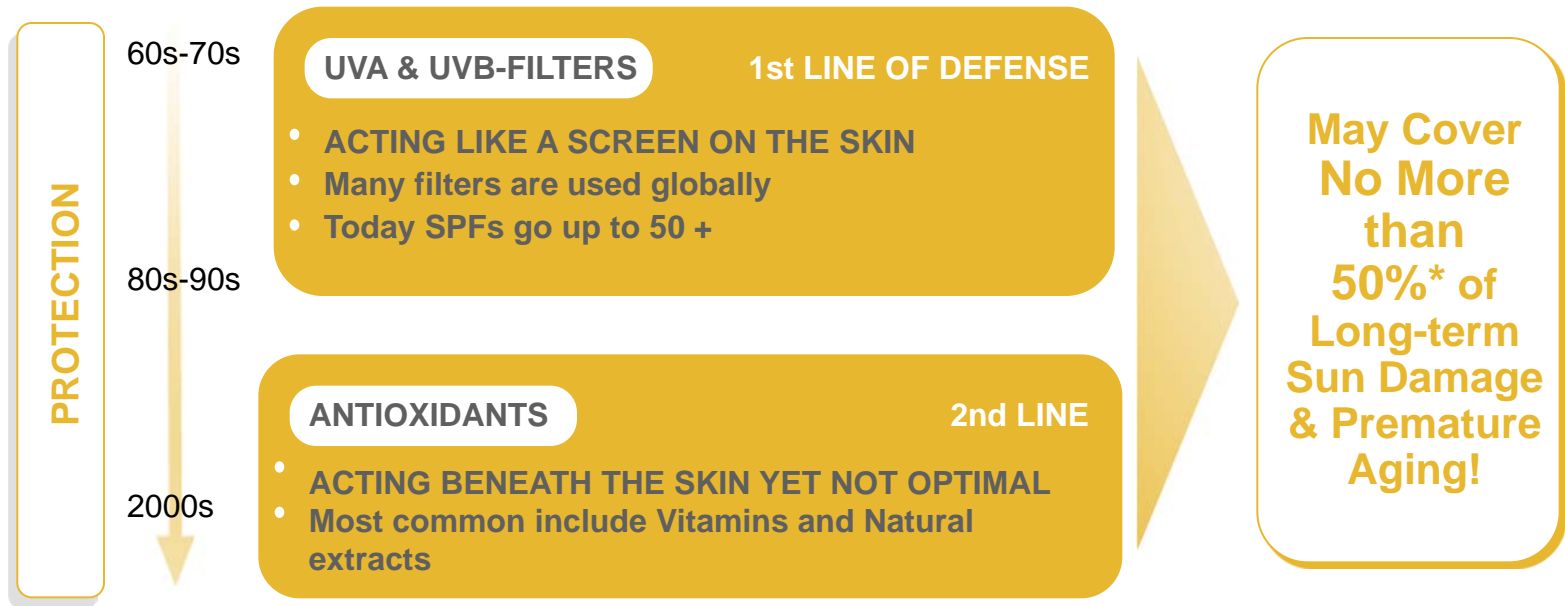
Cold Sore
(Herpes Simplex-1)

SUN CARE CONSUMERS EXPECTATIONS



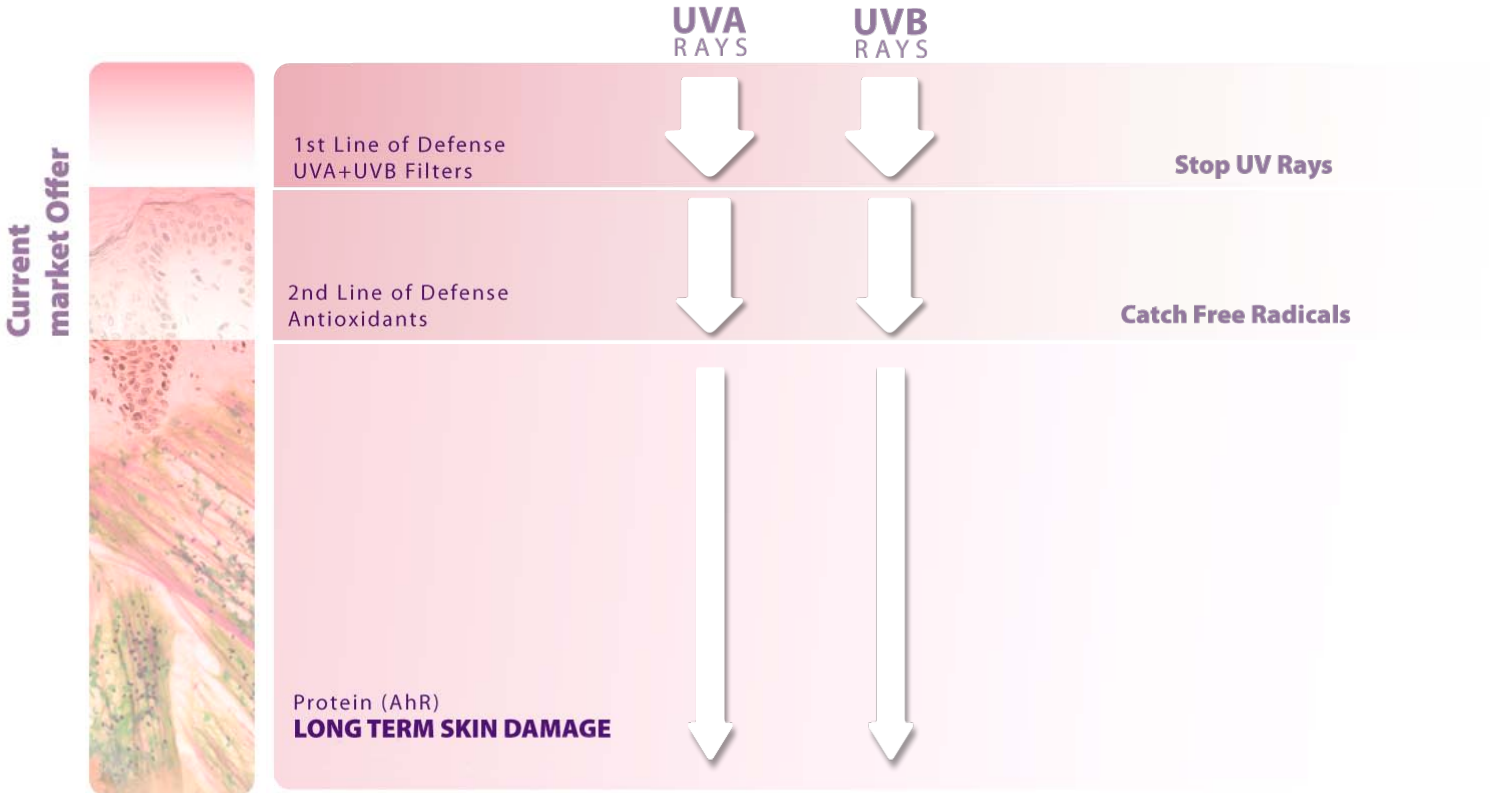
THE CURRENT MARKET

ANSWERS



*100% Protection is not achievable today

HOW DOES CURRENT PROTECTION WORK ?



UVB DAMAGE

NEW MECHANISM

- > Recent research (4 years ago) has identified a very important mechanism in UVB-induced cell damage: the Arylhydrocarbon Receptor (AhR).
- > UVB rays cause a toxic response in the skin via activation of the AhR.
- > According to some dermatological experts, this accounts for at least 50% of the induction of damage, including skin cancer and premature aging.
- > Use of traditional formulations containing UV filters as a means to attenuate the UV radiation are insufficient to prevent this from happening, as not all of the UV rays are prevented from penetrating into the skin.

UVB DAMAGE

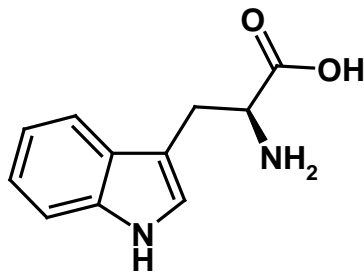
NEW MECHANISM

- > The AhR is a protein complex found in the cytoplasm of every cell in all vertebrates. Its primary function is thought to be the metabolism of chemical species, either from food or from external sources. Its mechanism of action has been mostly studied with the toxicity of dioxin.
- > The AhR typically binds flat aromatic hydrocarbons, and in many cases makes them toxic via transportation from the cytoplasm into the nucleus where they interact with DNA.

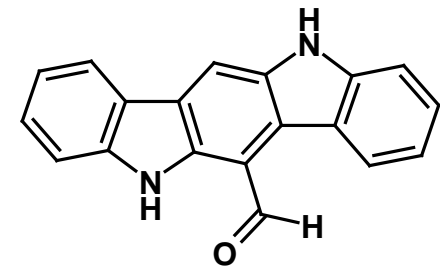
ARYLHYDROCARBON RECEPTOR

UVB PATHWAY

- > The essential amino acid **Tryptophan** absorbs UVB rays and forms a photoproduct which interacts with the AhR
- > Its photoproduct is called FICZ (6-Formylindol[3,2 b]carbazole)



Tryptophan

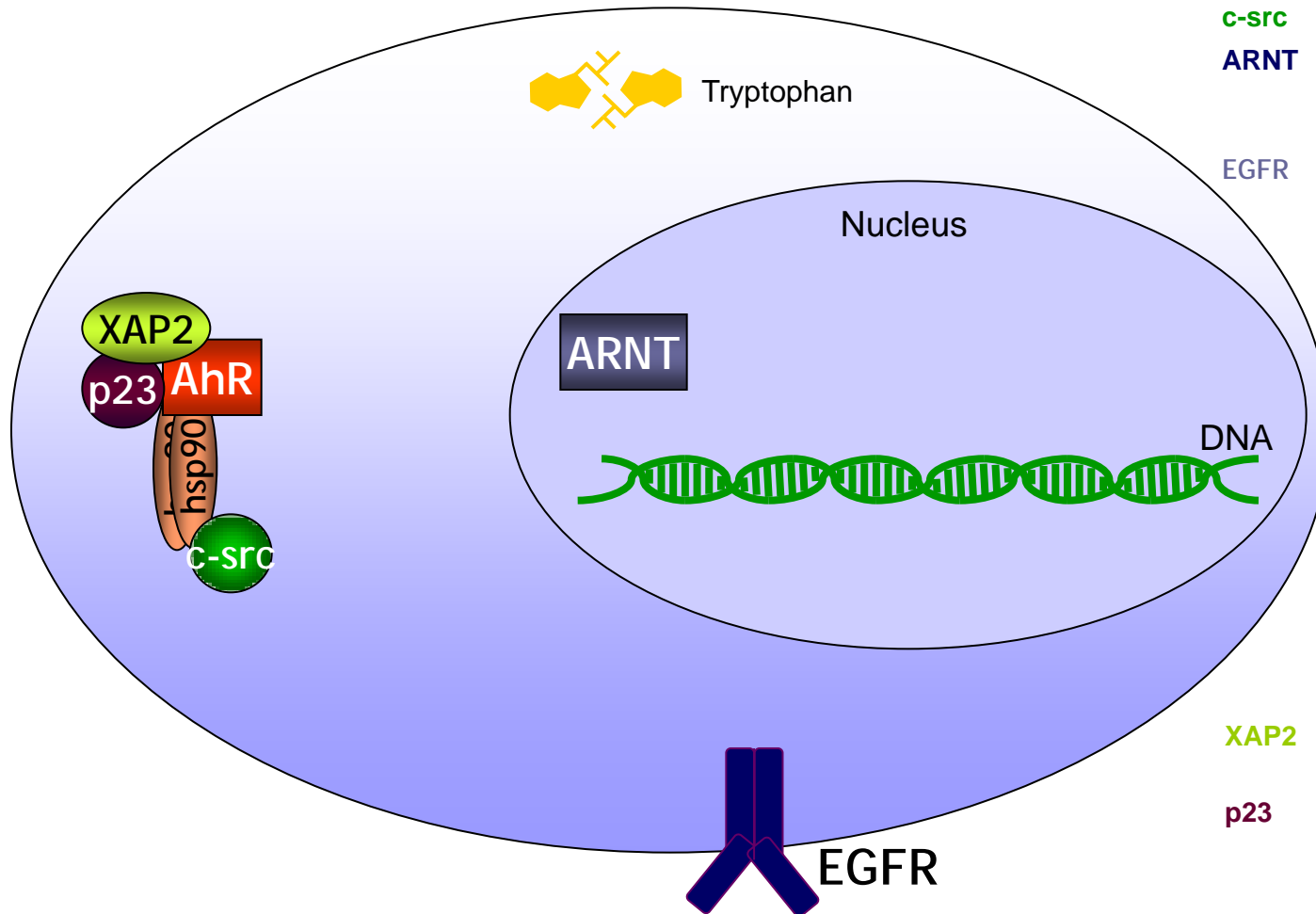


FICZ

ARYLHYDROCARBON RECEPTOR

UVB PATHWAY

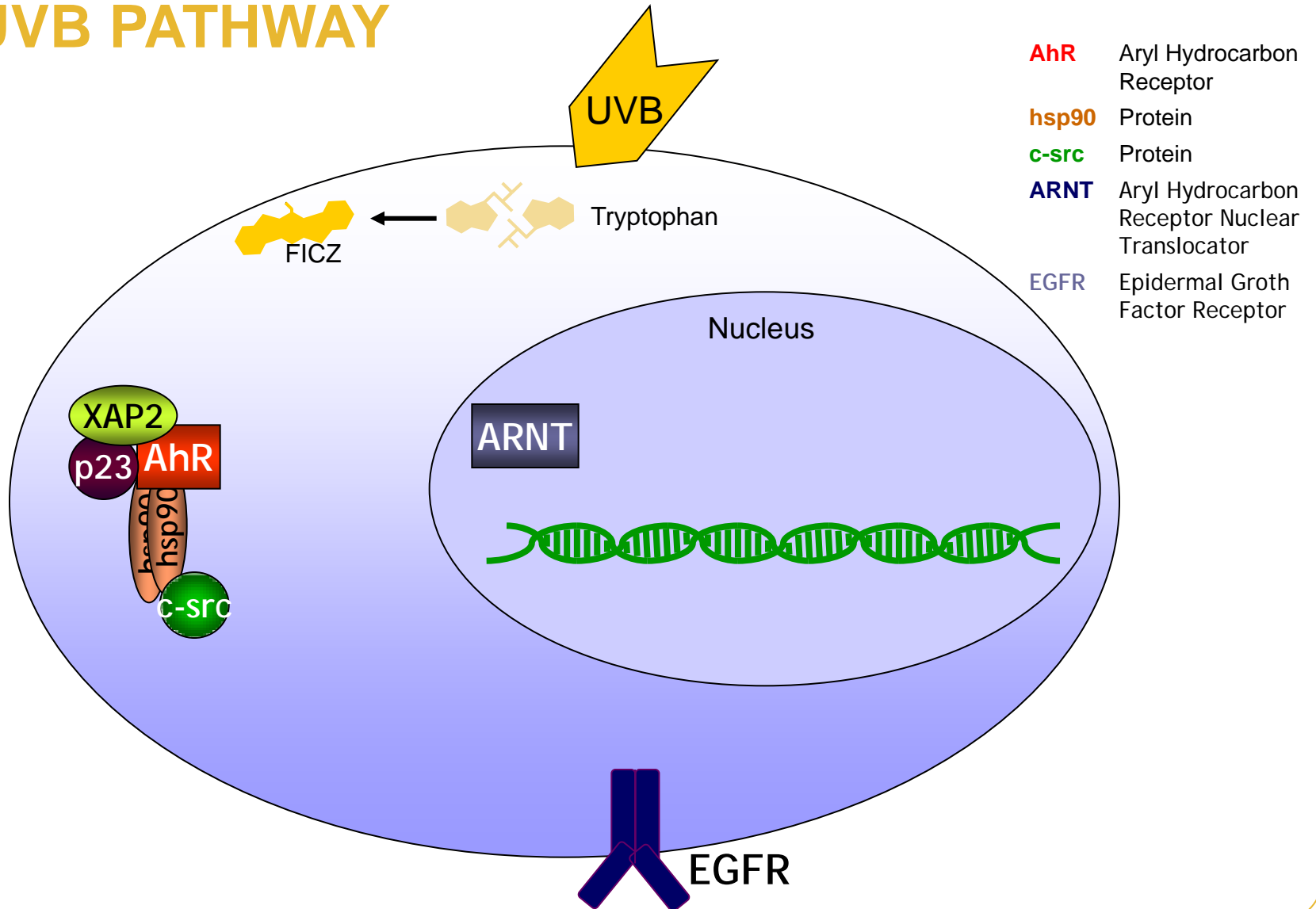
AhR Aryl Hydrocarbon Receptor
hsp90 Protein
c-src Protein
ARNT Aryl Hydrocarbon Receptor Nuclear Translocator
EGFR Epidermal Groth Factor Receptor



XAP2 Immunophilin-like Protein
p23 hsp90 Chaperone

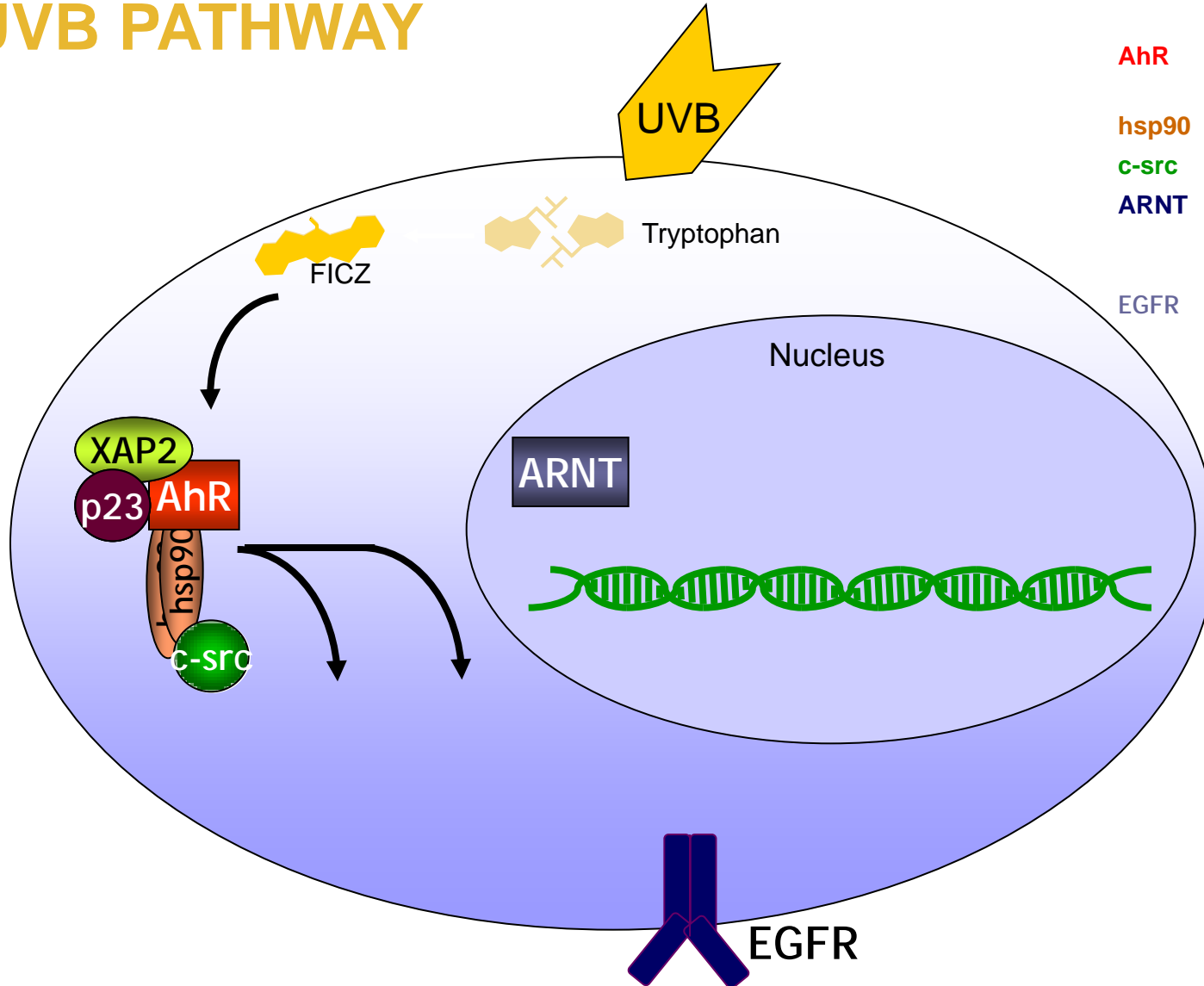
ARYLHYDROCARBON RECEPTOR

UVB PATHWAY



ARYLHYDROCARBON RECEPTOR

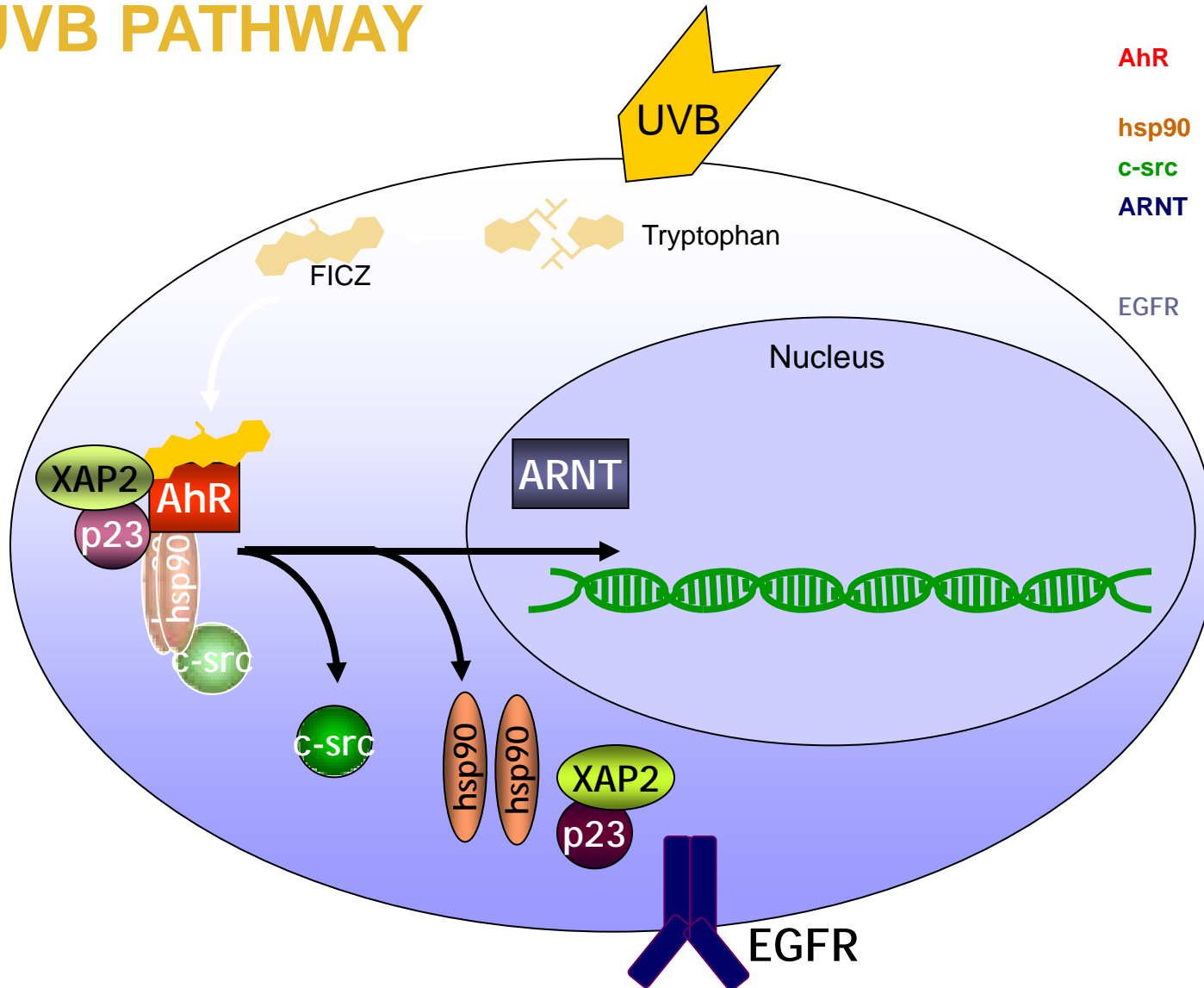
UVB PATHWAY



- AhR** Aryl Hydrocarbon Receptor
- hsp90** Protein
- c-src** Protein
- ARNT** Aryl Hydrocarbon Receptor Nuclear Translocator
- EGFR** Epidermal Groth Factor Receptor

ARYLHYDROCARBON RECEPTOR

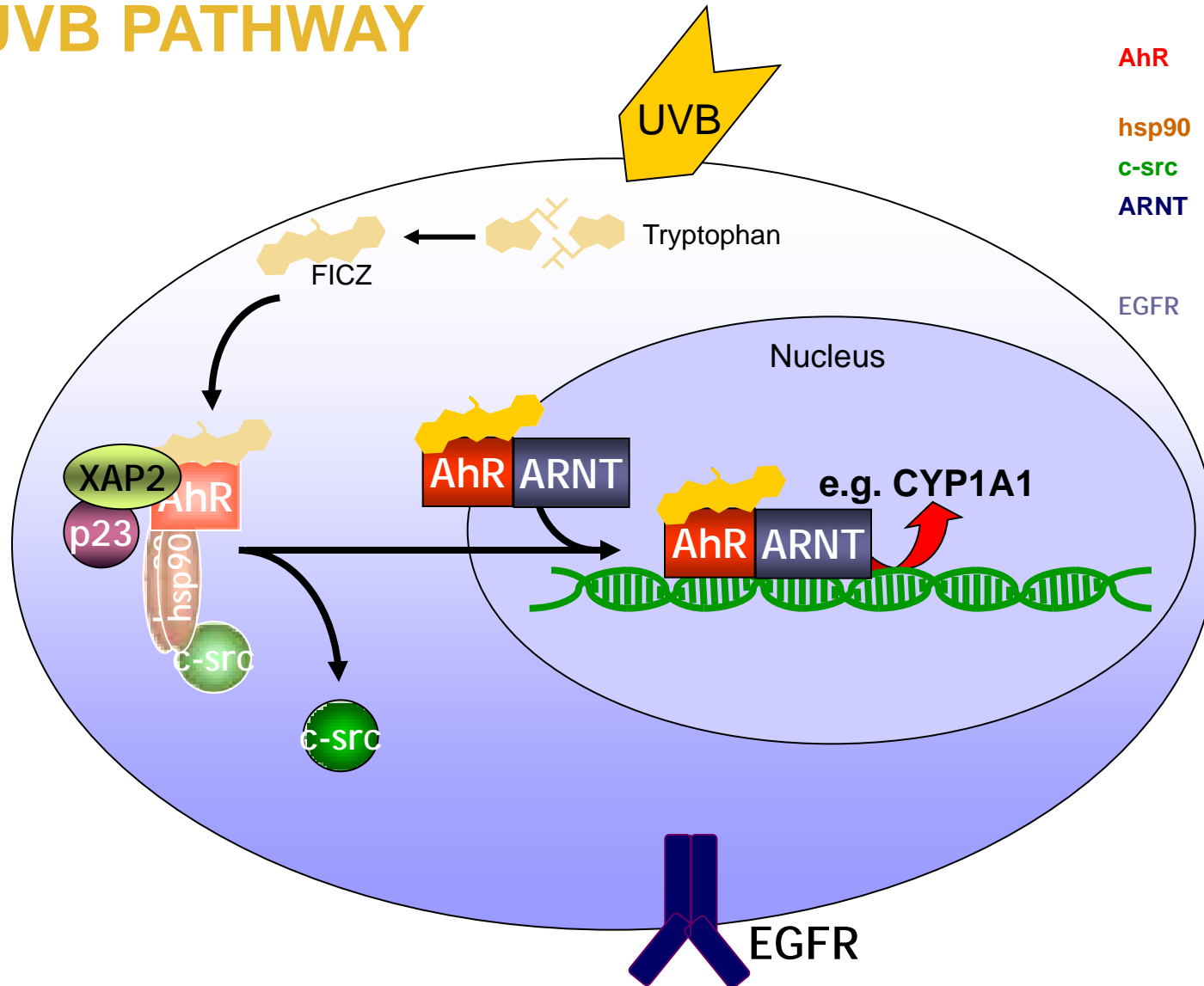
UVB PATHWAY



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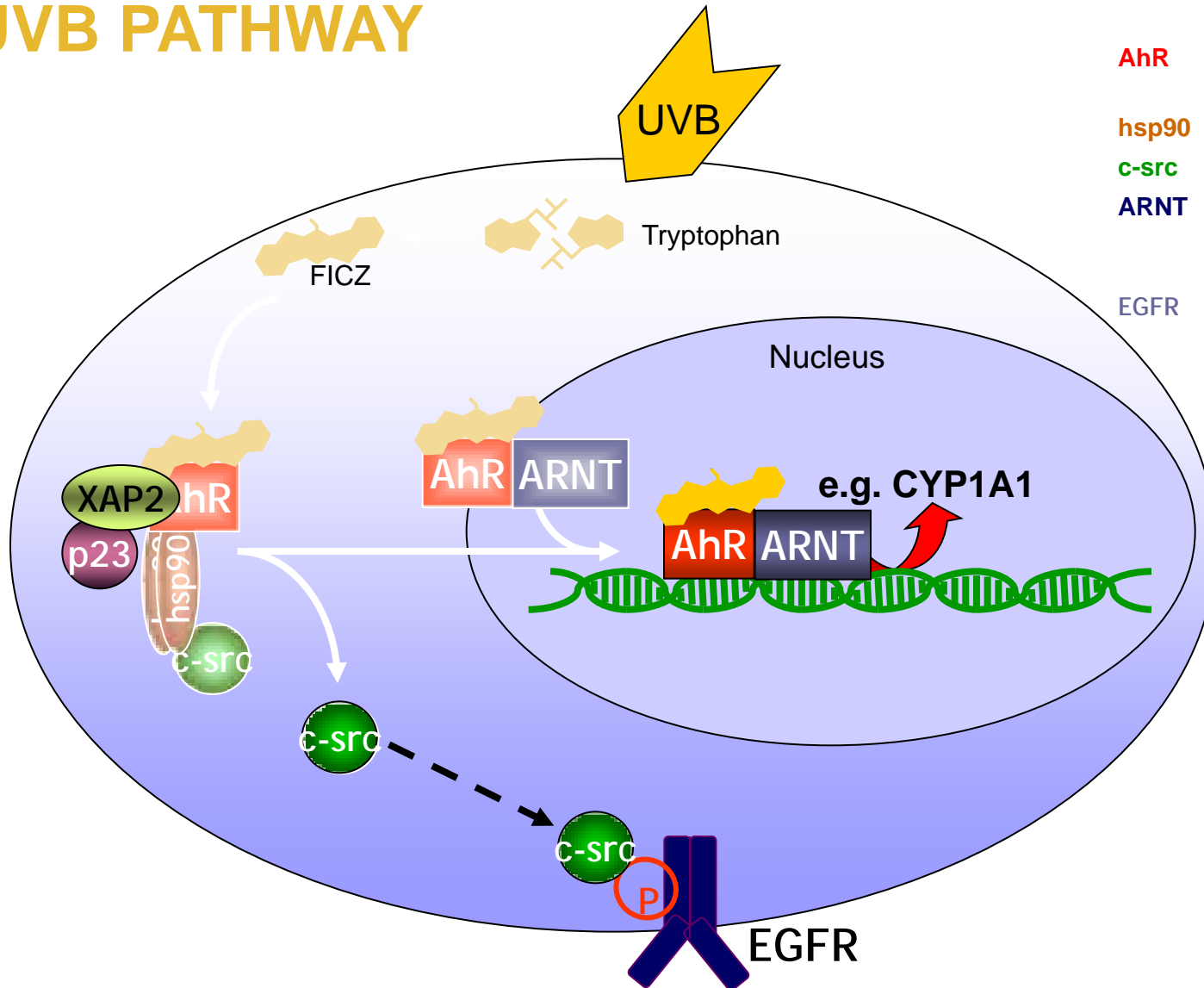
UVB PATHWAY



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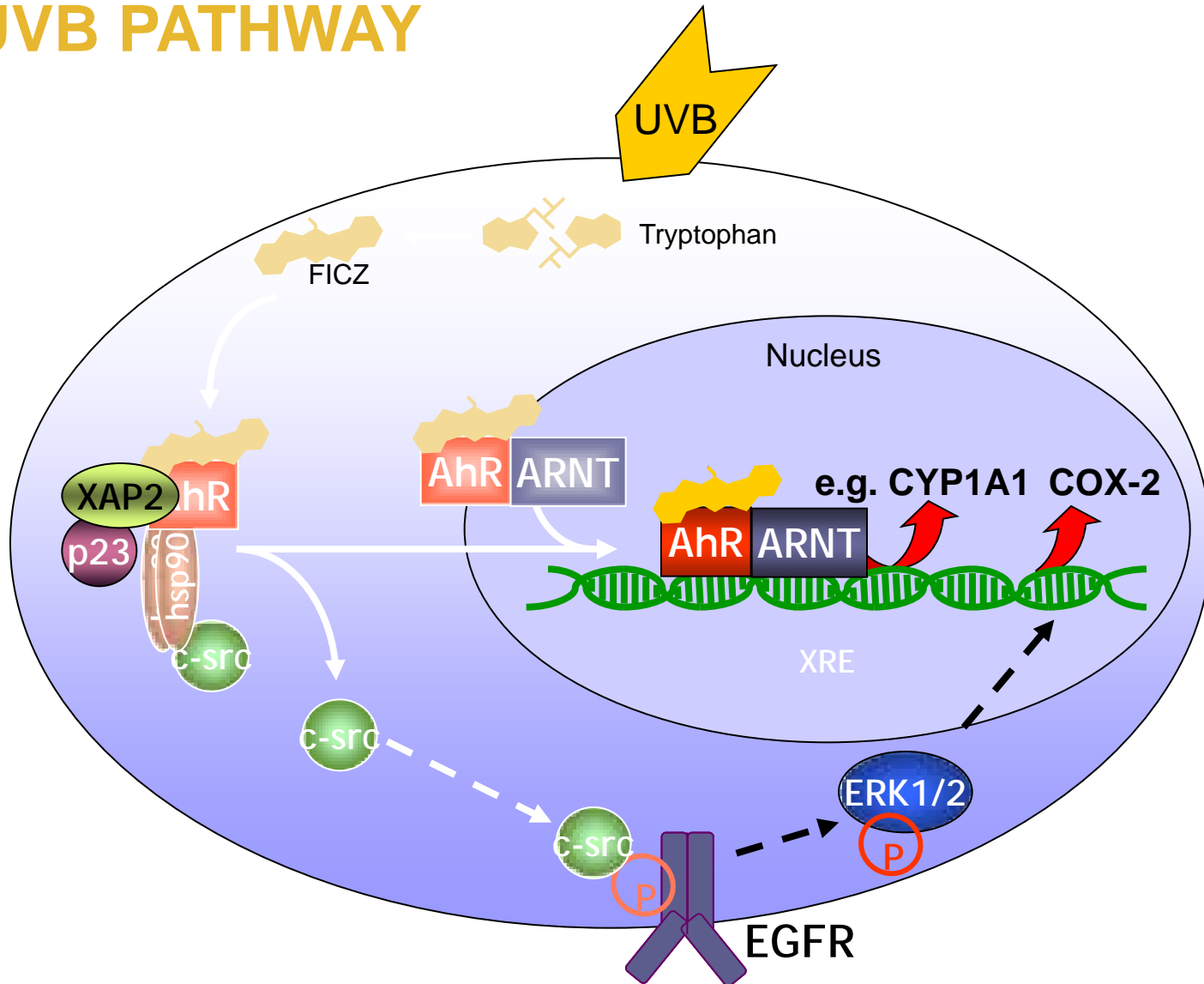
ARYLHYDROCARBON RECEPTOR

UVB PATHWAY



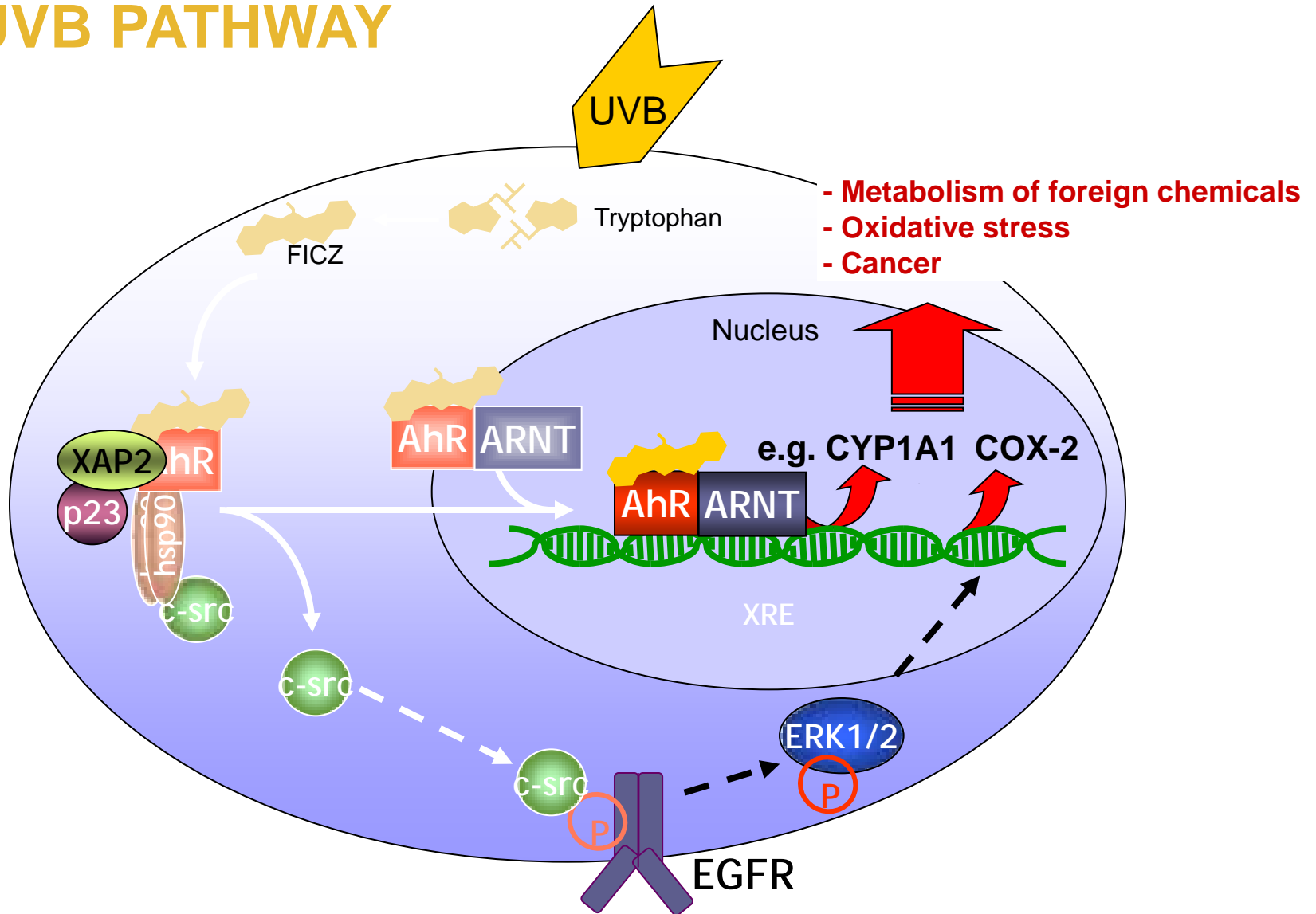
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ARYLHYDROCARBON RECEPTOR UVB PATHWAY



ARYLHYDROCARBON RECEPTOR

UVB PATHWAY

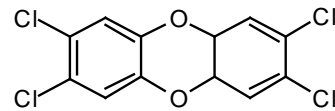


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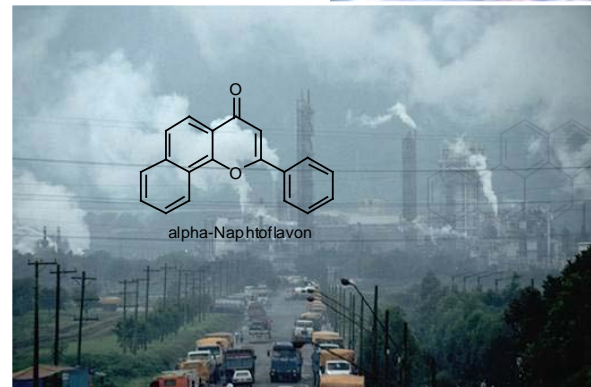
CHEMICAL INTERACTIONS

Chemicals that bind to the AhR are flat polyaromatic hydrocarbons e.g.

- > 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)
- > Benz[a]pyrene (B[a]P), a constituent of tobacco smoke and automobile exhaust
- > Dibenzofurans, a constituent of tobacco smoke, creosote (which is used to preserve wood), coal tars
- > Polychlorinated Biphenyls were found in, electrical transformers, coal tars, plastic additives for cable insulation, etc. These very toxic substances are no longer allowed to be used.
- > Other industrial pollutants found in smog etc



2,3,7,8-Tetrachlorodibenzodioxin





AhR ANTAGONISTS

FOR UVB AND TOXIN PROTECTION

A REVOLUTIONARY strategy for UVB protection:

Inhibition of AhR activation in the skin

Enhanced protection against:

- **Photo aging (in combination with UV filters and antioxidants)**
- **Damage caused by environmental toxins**
- **Inflammation and immunosuppression**
- **Photocarcinogenesis**

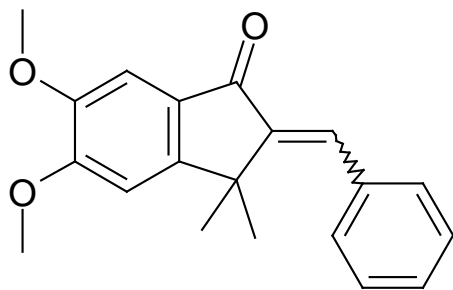
AhR ANTAGONISTS

SCREENING

We developed and screened cosmetically acceptable candidates to reversibly block the AhR from interacting with FICZ and thereby significantly reduce the production of the CYP1A1, COX-2 and other enzymes in the nucleus.

The blocker or antagonist must bind to the AhR in place of FICZ and prevent it from being transported into the nucleus.

AhR ANTAGONIST



Patent Application
WO2007/128723

Chemical Name:

2-Benzylidene-5,6-dimethoxy-3,3-dimethylindan-1-one
(mixture of E & Z isomers)

INCI:

Benzylidene Dimethoxydimethylindanone



Efficacy Studies

A whole battery of *in vitro* studies, commencing with HaCaT keratinocytes, progressing onto primary keratinocytes and then studying the effect of finished products in 3D skin models to show the efficacy of Benzylidene Dimethoxydimethylindanone have been undertaken.

These were followed with an *in vivo* study of a finished formulation containing Benzylidene Dimethoxydimethylindanone on a human panel.

This work was a collaboration between Professor Jean Krutmann and Dr. Fritsche of the Institute of Environmental Medical Research at the University of Düsseldorf, Germany.



Efficacy Studies

in vitro



EFFICACY STUDIES

IN VITRO

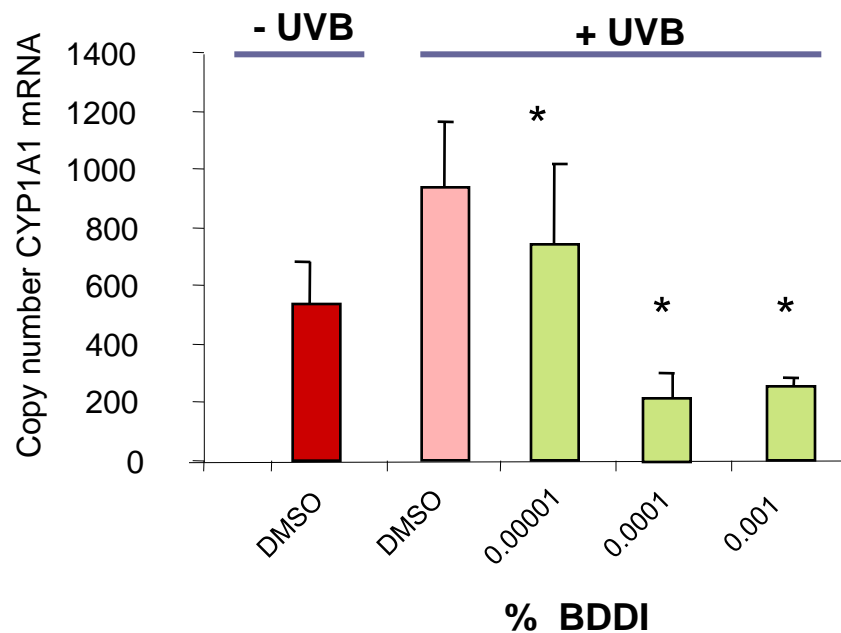
1. Inhibition of CYP1A1 in keratinocytes after UVB irradiation
2. Inhibition of CYP1A1 in keratinocytes in the presence of B[a]P
3. Inhibition of COX-2 in keratinocytes after UVB irradiation
4. Fluorescent staining to show inhibition of AhR shuttling into the nucleus
5. Inhibition of CYP1A1 in 3D skin models after UVB irradiation by BDDI in a finished formulation

AhR ANTAGONIST

CYP1A1 FROM UVB

Method

1. Incubation of HaCaT keratinocytes for 1h with BDDI or 0.1 % DMSO
2. Irradiation with 100 J/m² UVB
3. Further incubation for 4 h
4. Preparation of mRNA and quantification of *CYP1A1*



BDDI is a potent inhibitor of UVB-induced CYP1A1 expression in keratinocytes.

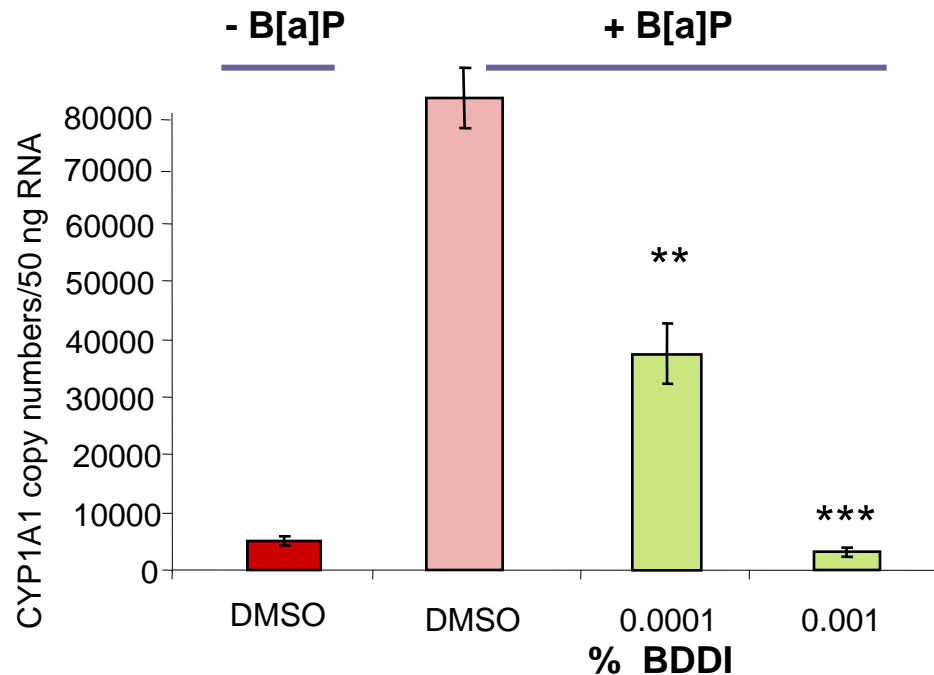
* P < 0.05

AhR ANTAGONIST

CYP1A1 FROM B[a]P

Method

1. Incubation of normal human primary keratinocytes (NHEK) for 1 h with BDDI or 0.1 % DMSO
2. Incubation for 16 h with 250 nM benz[a]pyrene
3. Preparation of mRNA and quantification of *CYP1A1*



BDDI is a potent inhibitor of B[a]P-induced CYP1A1 expression

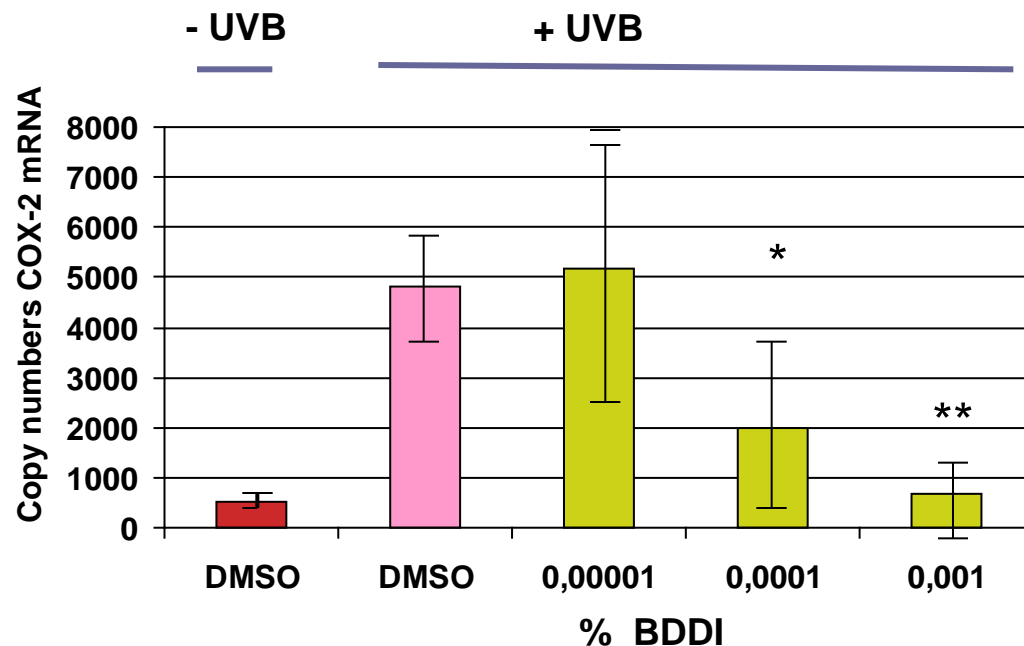
** $p < 0.01$
*** $p < 0.001$

AhR ANTAGONIST

COX-2 FROM UVB

Method

1. Incubation of HaCaT keratinocytes for 1h with BDDI or 0.1 % DMSO
2. Irradiation with 100 J/m² UVB
3. Further incubation for 4 h
4. Preparation of mRNA and quantification of COX-2



BDDI is a potent inhibitor of UVB-induced COX-2 expression

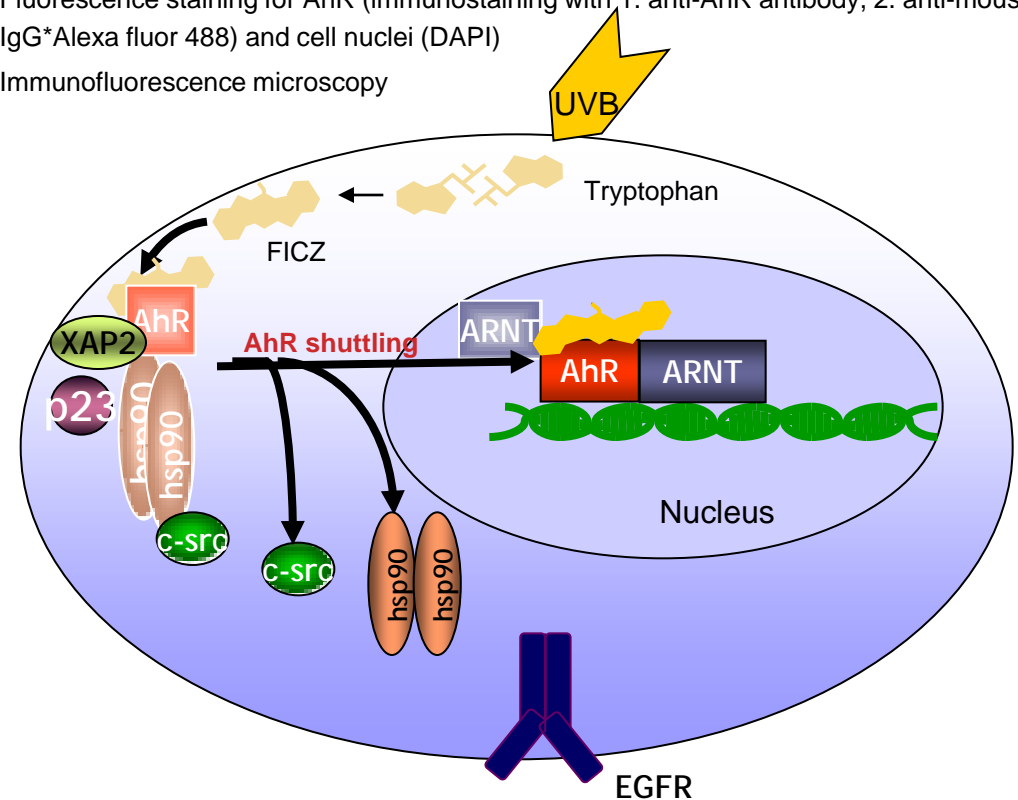
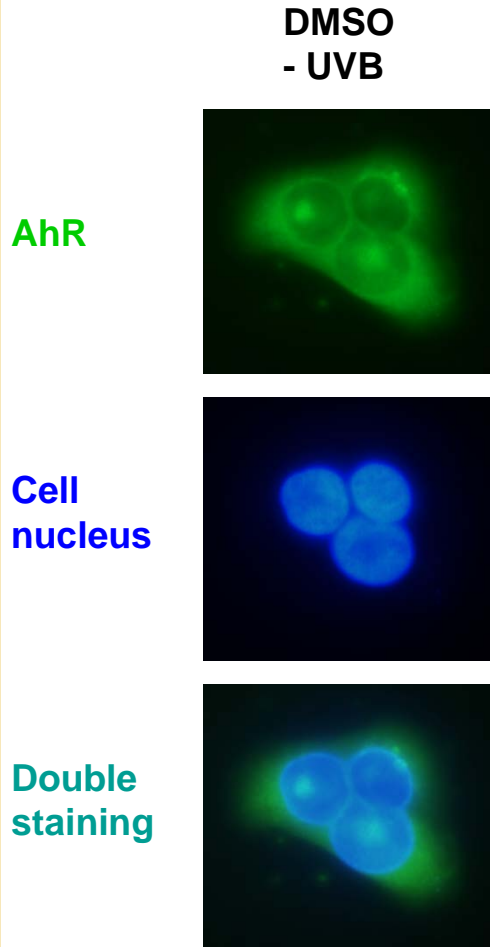
* $p < 0.05$
** $p < 0.01$

AhR INHIBITION

FLUORESCENT STAINING

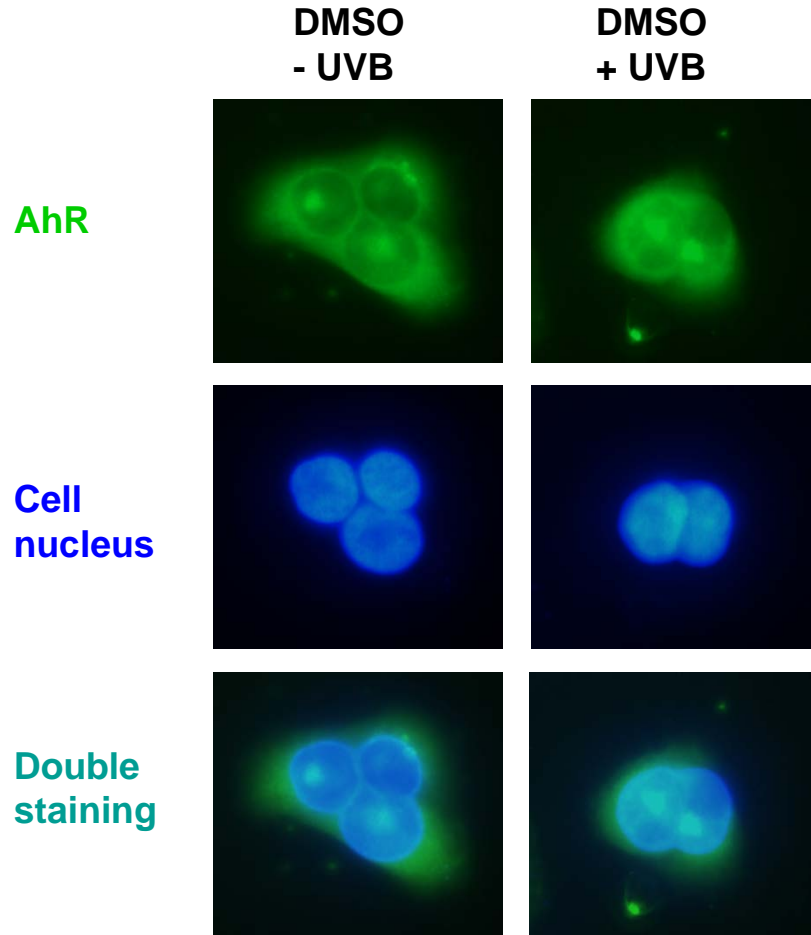
Method

1. Incubation of HaCaT keratinocytes for 1 h with 0.0001 % BDDI or 0.0003 % MNF
2. Irradiation with 100 J/m² UVB or 40 pM FICZ
3. Further incubation for 40 min
4. Cell fixation
5. Fluorescence staining for AhR (immunostaining with 1. anti-AhR antibody, 2. anti-mouse IgG*Alexa fluor 488) and cell nuclei (DAPI)
6. Immunofluorescence microscopy



AhR INHIBITION

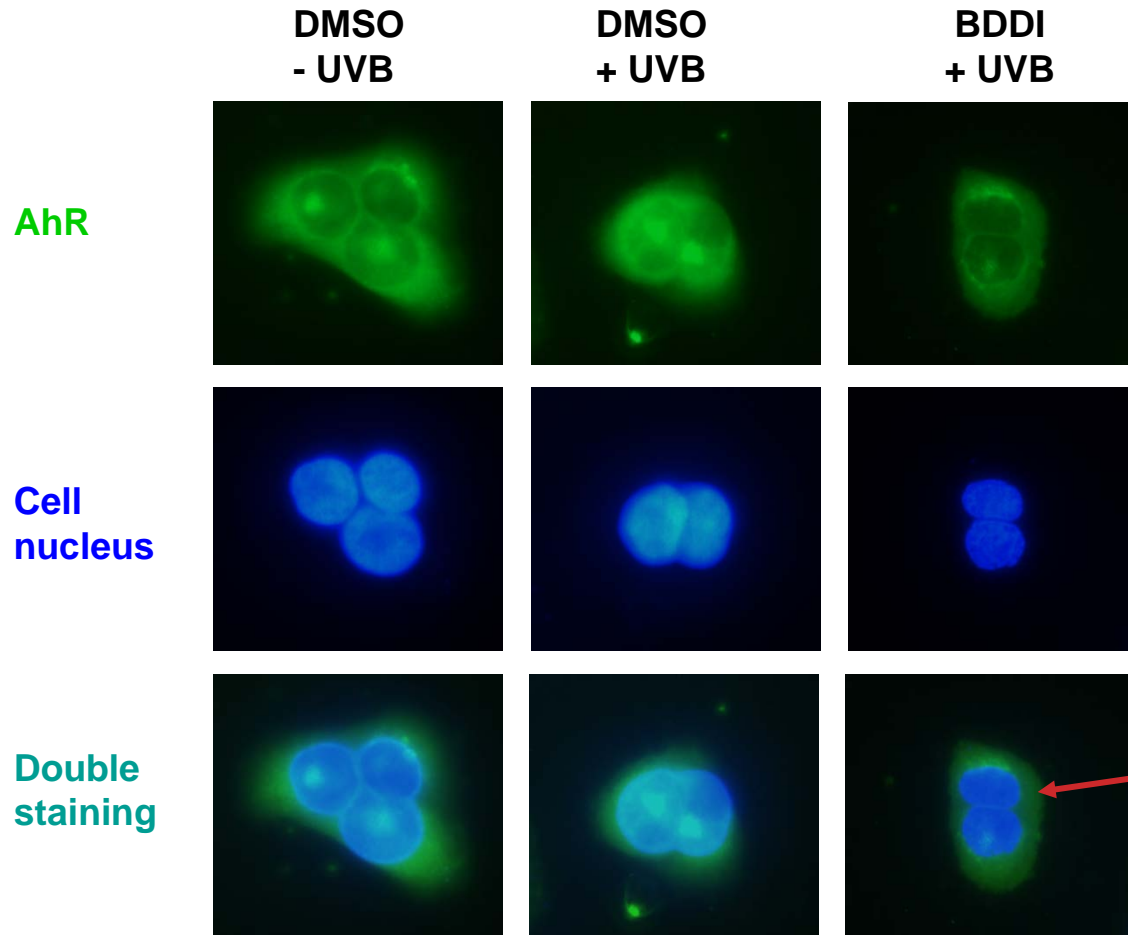
FLUORESCENT STAINING



UVB induces
increased nuclear
staining for AhR

AhR INHIBITION

FLUORESCENT STAINING



UVB induces
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staining for AhR

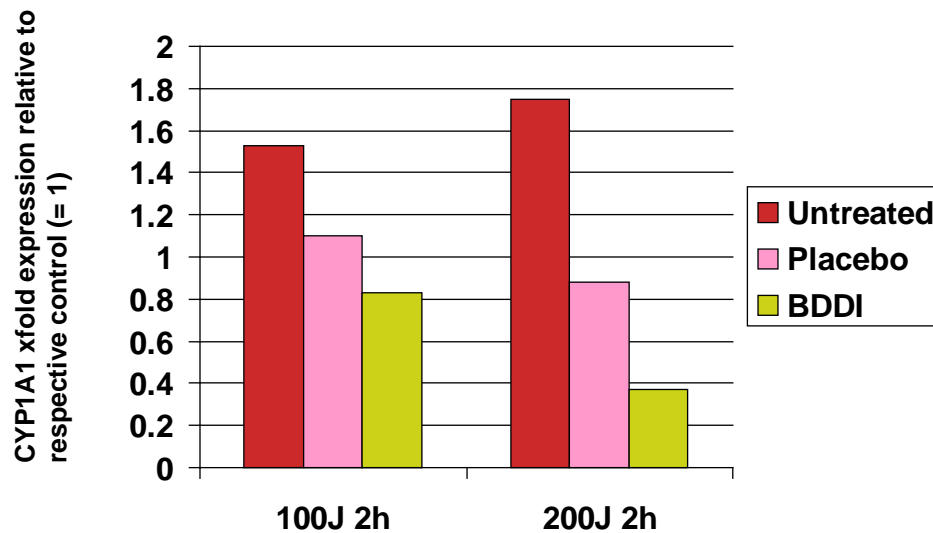
Inhibition of UVB-induced
nuclear staining by BDDI

AhR ANTAGONIST

CYP1A1 INHIBITION – 3D Skin Model

Method

1. Incubation of Epiderm models with hydrodispersion gel with 0.05 % BDDI for 24h
2. Irradiation with 100 J/m² or 200 J/m² UVB
3. Further incubation for 2 h
4. Preparation of mRNA and quantification of *CYP1A1*



BDDI is a potent inhibitor of UVB-induced CYP1A1 expression in *3D epidermis models*



Efficacy Studies

in vivo

AhR ANTAGONIST

TOXIN METABOLISM AND AGING MARKERS

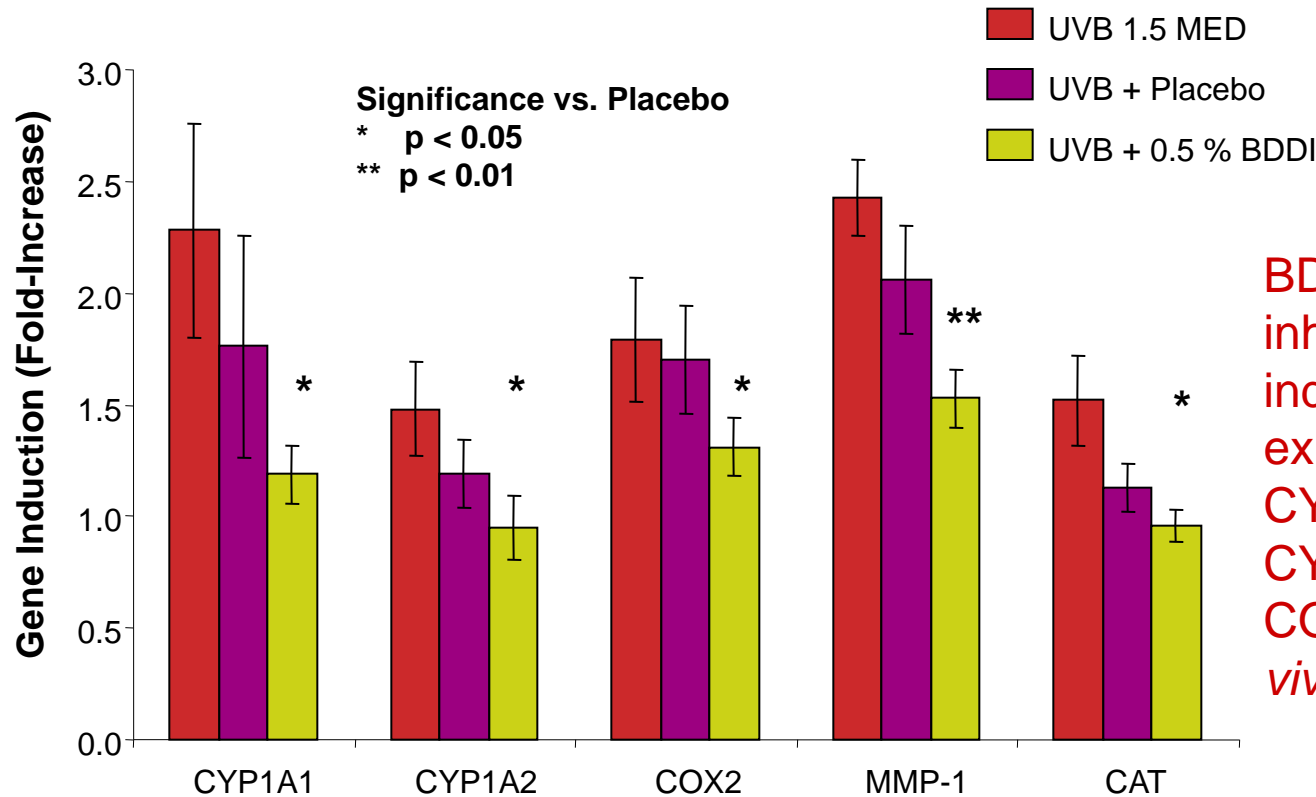
Method

1. Daily treatment of 10 human volunteers with a hydrodispersion gel with 0.5 % BDDI (2 mg/cm²) for 4 days (area: buttock skin, size: 16 cm²)
2. On day 4, 2h after the daily treatment: irradiation with UVB (1.5 MED, Dermalight 80 max. 306 nm, Hönle Medizintechnik GmbH, Kaufering, D)
3. After 24h, 4 mm punch biopsies were taken from the following areas:
 - Non-irradiated control (no UVB)
 - UVB-irradiated
 - Pre-treated with placebo, UVB-irradiated
 - Pre-treated with formula containing 0.5 % BDDI, UVB irradiated
4. Preparation and quantification of mRNA for the following markers which are typically induced by UVB:
 - CYP1A1, CYP1A2: foreign chemical and drug metabolism
 - MMP-1: skin aging
 - COX-2: inflammation
 - CAT: oxidative stress

AhR ANTAGONIST

TOXIN METABOLISM AND AGING MARKERS

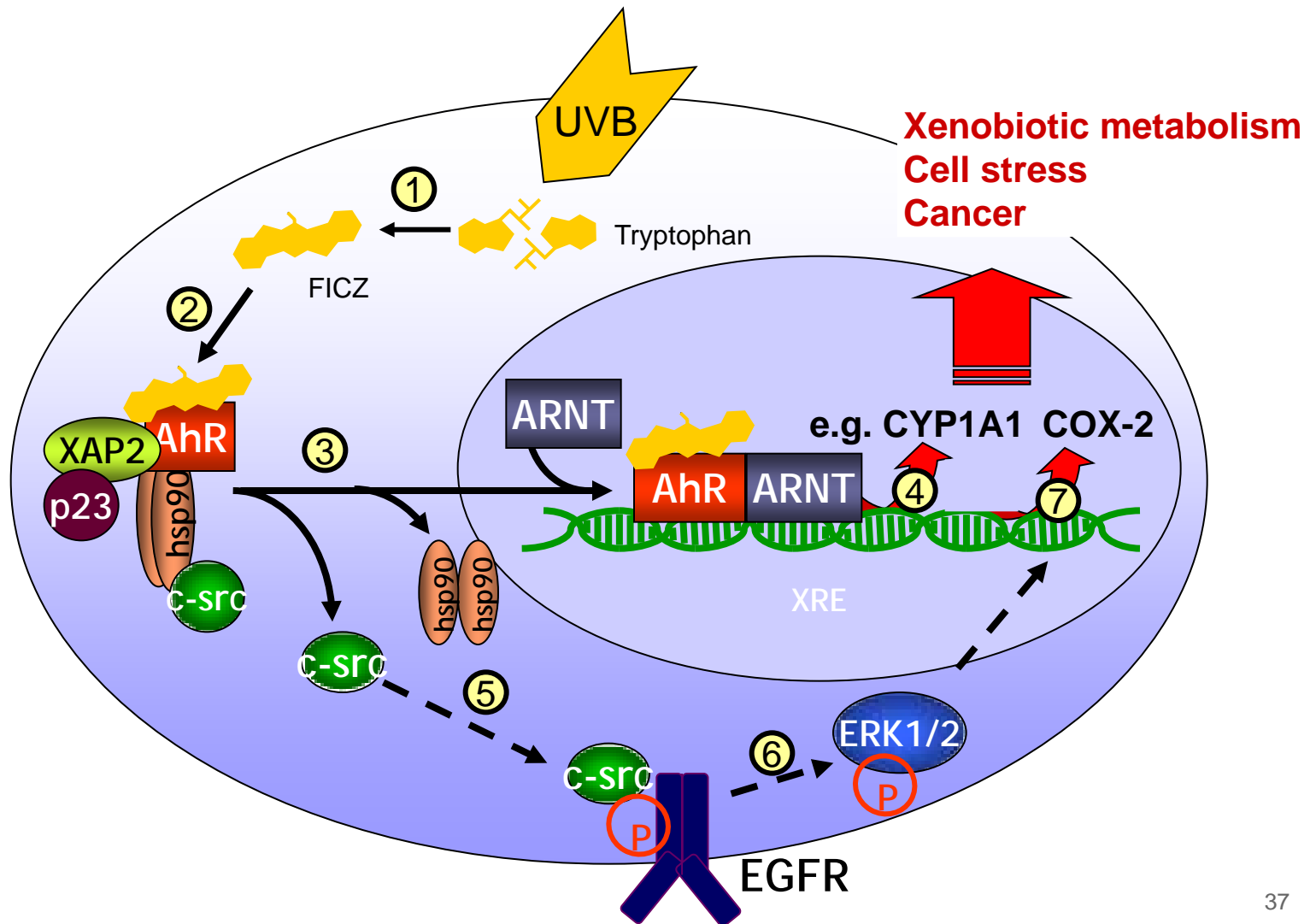
Results



BDDI significantly inhibits UVB-induced expression of CYP1A1, CYP1A2, MMP-1, COX-2 and CAT *in vivo*

ARYLHYDROCARBON RECEPTOR

UVB PATHWAY BEFORE

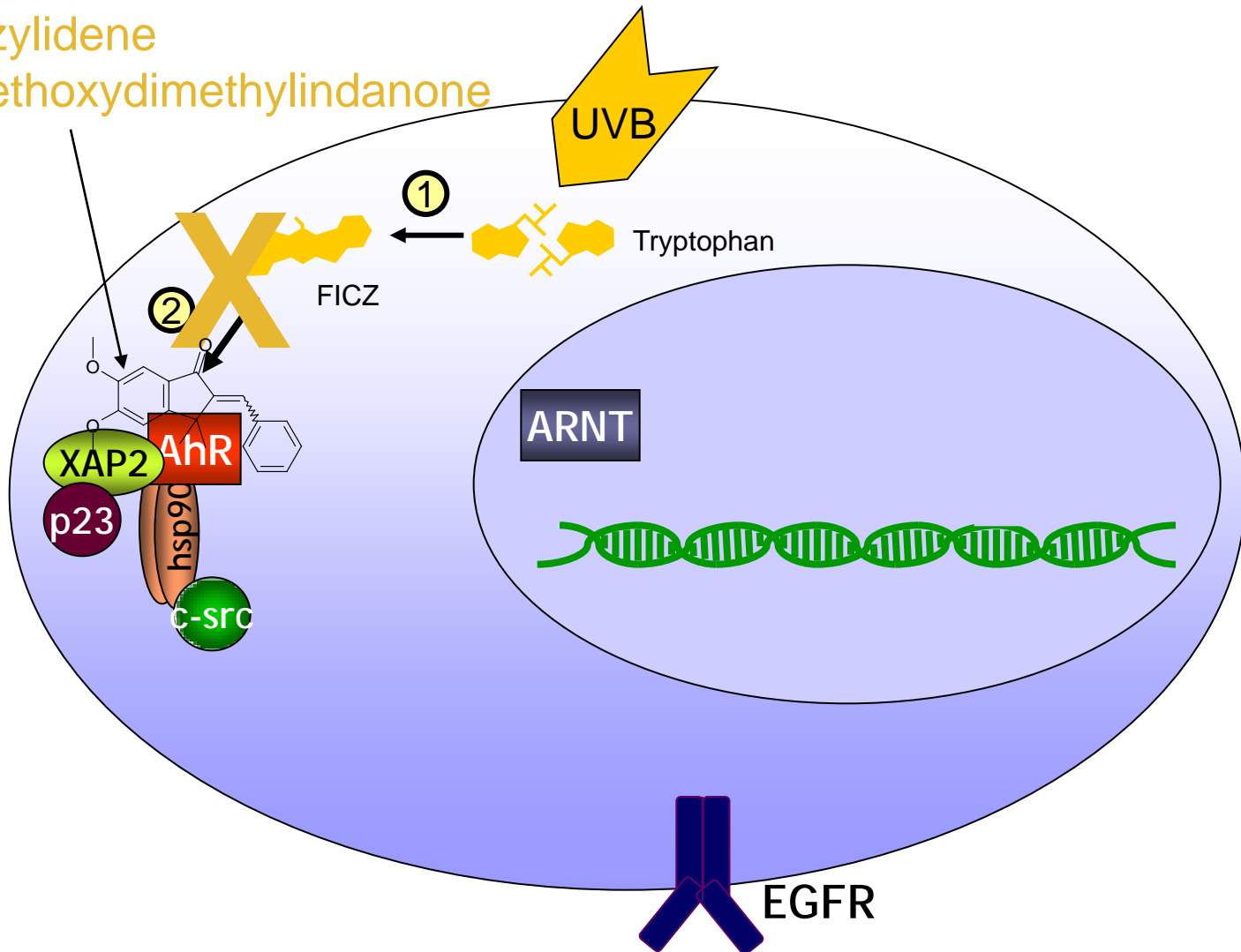


ARYLHYDROCARBON RECEPTOR

UVB PATHWAY WITH

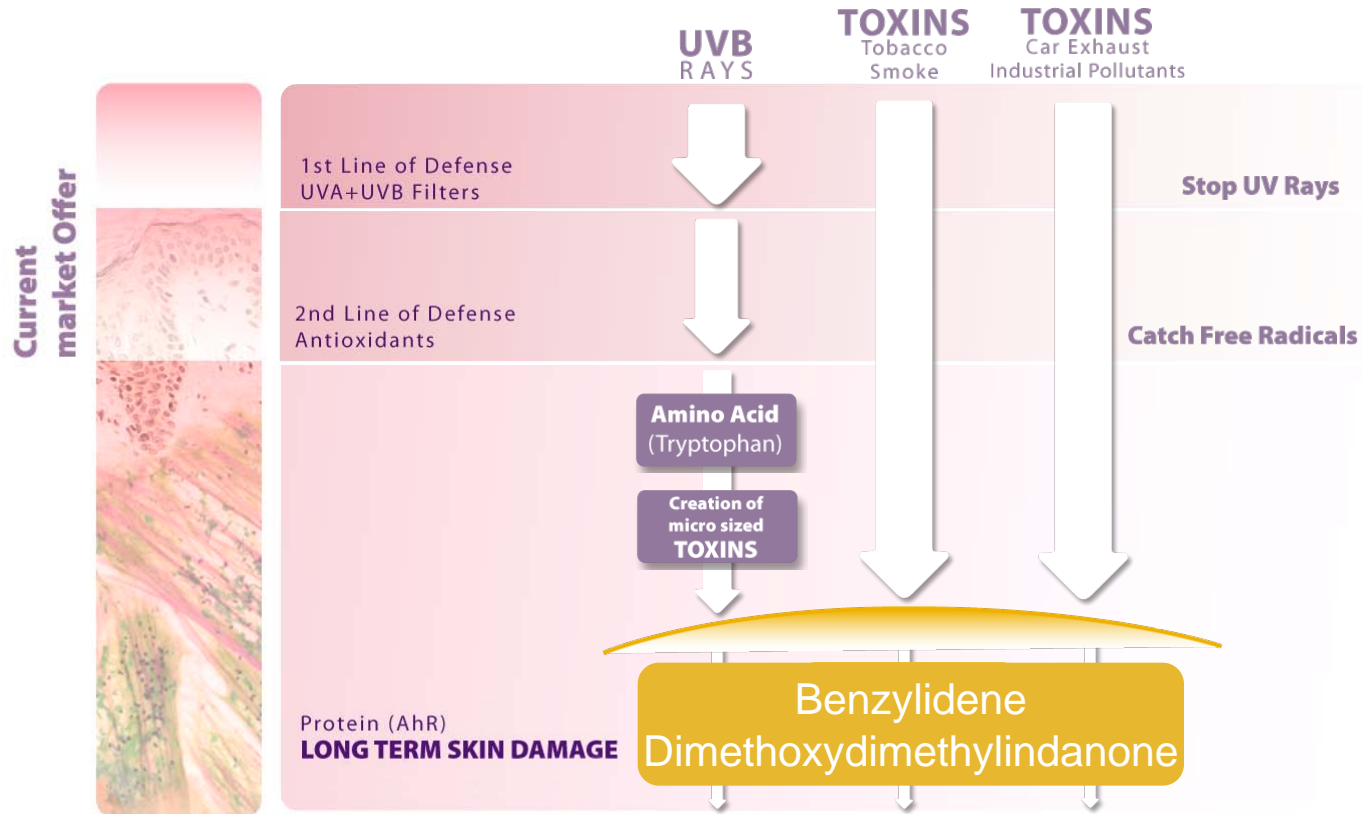
Benzyldiene

Dimethoxydimethylindanone



HOW DOES IT WORK ?

Benzylidene Dimethoxydimethylindanone
is the 1st Toxin Neutralizer, going beyond 50% protection!



AhR ANTAGONIST

SUMMARY

- BDDI is the first toxin neutralizer which:
 - Works as an advanced cellular shield, optimizing beyond the 2nd layer of defense
 - Offers superior UVB and toxin protection
 - Promotes cell self-defense from:
 - Sun-induced aging
 - Pollution-induced aging

- Promotes skin health and beauty from within
 - Goes Beyond Sunscreen – Offers ultimate sun protection from the inside
 - Neutralizes Daily Stressors – Offers ultimate daily protection from the inside



Gabriele Vielhaber



Oskar Koch



Jean Krutmann

> Acknowledgments

THANK
YOU

APPENDIX



EFFICACY STUDIES

IN VITRO

1. Inhibition of CYP1A1 in HaCaT keratinocytes after UVB irradiation
2. Inhibition of CYP1A1 in HaCaT keratinocytes in the presence of FICZ
3. Inhibition of COX-2 in HaCaT keratinocytes after UVB irradiation
4. Inhibition of CYP1A1 in primary keratinocytes in the presence of B[a]P
5. Proof of reversibility of the inhibition in HaCaT after UVB irradiation*
6. Fluorescent staining to show inhibition of AhR shuttling into the nucleus by DB
7. Inhibition of CYP1A1 in 3D skin models after UVB irradiation by DB in a finished formulation

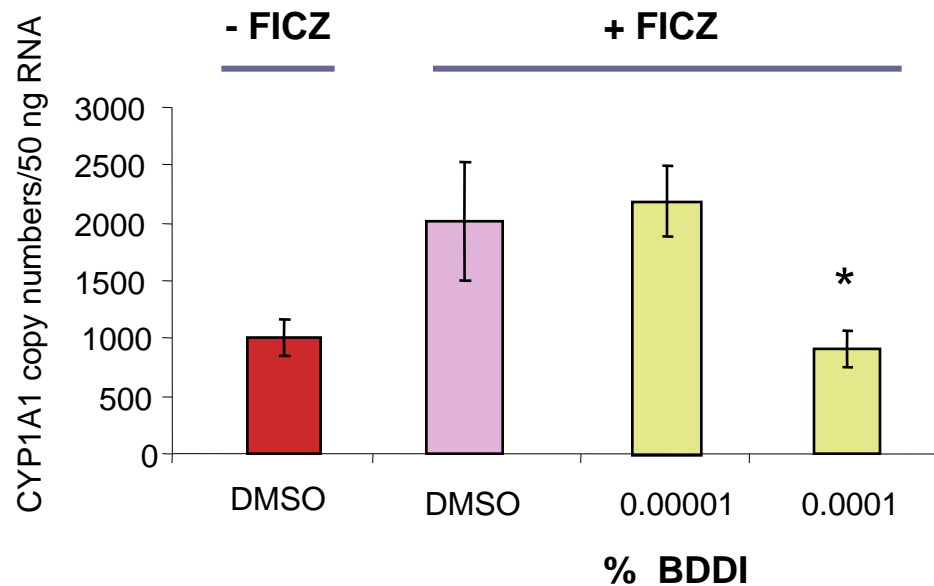
* based on CYP1A1 expression

AhR ANTAGONIST

FICZ

Method

1. Incubation of HaCaT keratinocytes for 1 h with BDDI or 0.1 % DMSO
2. Incubation for 1.5 h with 10 nM FICZ
3. Further incubation for 4 h
4. Preparation of mRNA and quantification of *CYP1A1*



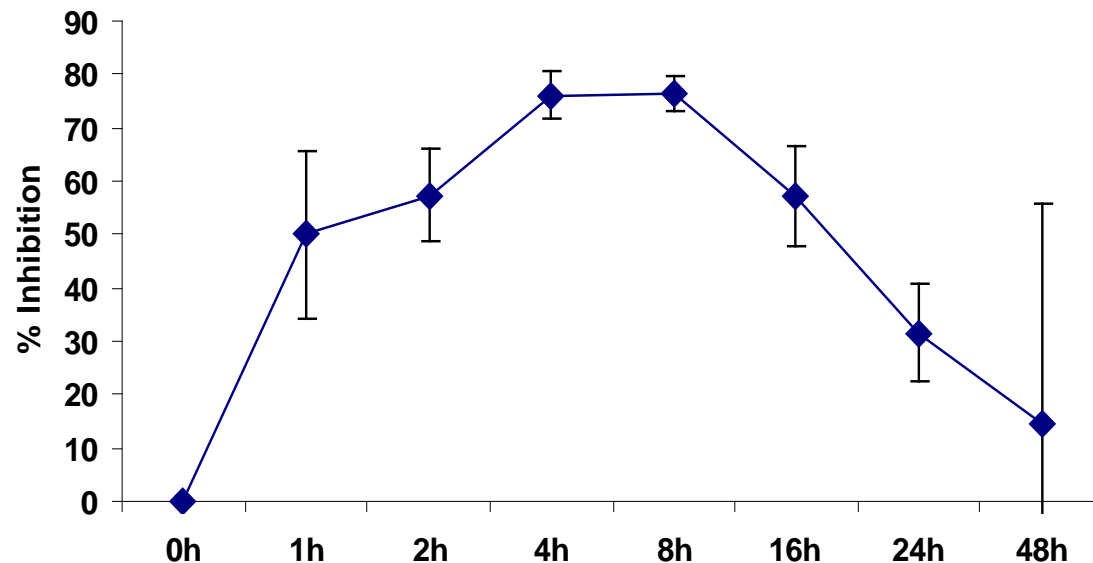
BDDI is a potent inhibitor of FICZ-induced CYP1A1 expression (* $p < 0.05$)

AhR ANTAGONIST

REVERSIBILITY

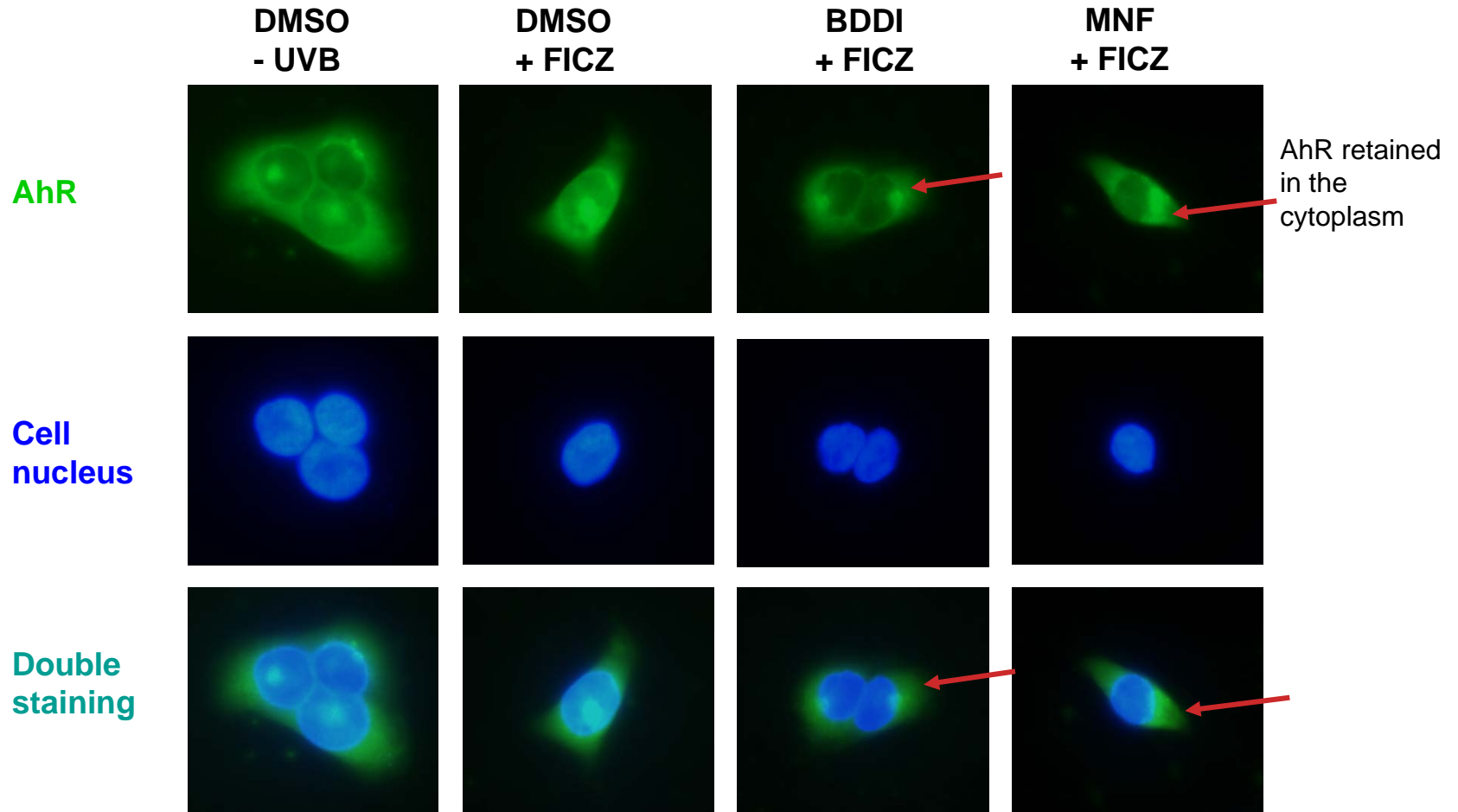
Method

1. Incubation of HaCaT keratinocytes for 0 – 48h with 0.0001 % BDDI Irradiation with 100 J/m² UVB
2. Further incubation for 4 h
3. Preparation of mRNA and quantification of *CYP1A1*



The inhibition of AhR by BDDI is reversible

AhR Inhibition_FICZ



**FICZ induces
increased nuclear
staining for AhR**

**Inhibition of FICZ-induced
nuclear staining by BDDI and
MNF**

AhR ANTAGONIST

3D skin model Formulation

	Raw material	INCI	Placebo w/w %	B w/w %
	H2O, demin.	Water (Aqua)	84,15	84,10
	SymDiol 68	1,2 Hexanediol, Caprylyl Glycol	0,60	0,60
	SymMoillent W/S	Trideceth-9, PEG-5 Isononanoate, Water	0,5	0,5
	Glycerin, 85%	Glycerin	1,00	1,00
	Hydrolite-5	1,2 Pentylene Glycol	1,00	1,00
B	PCL liquid 100	Cetearyl Ethylhexanoate	3,00	3,00
	Lanette O	Cetearyl Alcohol	2,00	2,00
	SymHelios® 1031	Benzylidene Dimethoxydimethylindanone	-	0,05
	Pemulen TR1	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0,20	0,20
	Ultrez-21	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0,05	0,05
	Mineral Oil 5°E	Mineral Oil	3,00	3,00
	Eutanol G	Octyldodecanol	3,50	3,50
	Abil 350	Dimethicone	0,50	0,50
c	Sodium Hydroxide, 10% sol.	Sodium Hydroxide	0,50	0,50
	Sum		100,00	100,00
	pH value		6,0	6,0

AhR ANTAGONIST

In-vivo test formulation

		INCI	Placebo	B
A.	Dragocid liquid	Phenoxyethanol, Methyl paraben, ethyl paraben, butyl paraben, propyl paraben, isobutyl paraben.	0,60	0,60
	Neo PCL ws. N	Trideceth-9, PEG-5 Ethylhexanoate	1,0	1,00
	H2O, de mineralised	Water (Aqua)	82,25	81,75
	Hydrolite-5	1,2 Pentylene Glycol	3,0	3,0
B.	PCL liquid 100	Cetearyl Ethylhexanoate	3,00	3,00
	Lanette O	Cetearyl Alcohol	2,00	2,00
	SymHelios® 1031	Benzylidene Dimethoxydimethylindanone		0,50
	Pemulen TR1	Acrylates/C10-30 Alkyl Acrylate Crosspolymer	0,25	0,25
	Paraffin oil 5°E	Mineral Oil	3,00	3,00
	Eutanol G	Octyldodecanol	4,00	4,00
	Abil 350	Dimethicone	0,50	0,50
C.	Sodium Hydroxide 10%	Sodium Hydroxide	0,40	0,40
	Sum		100,00	100,00
	pH		6,1	6,1

AhR ANTAGONIST

TOXIN METABOLISM AND AGING MARKERS

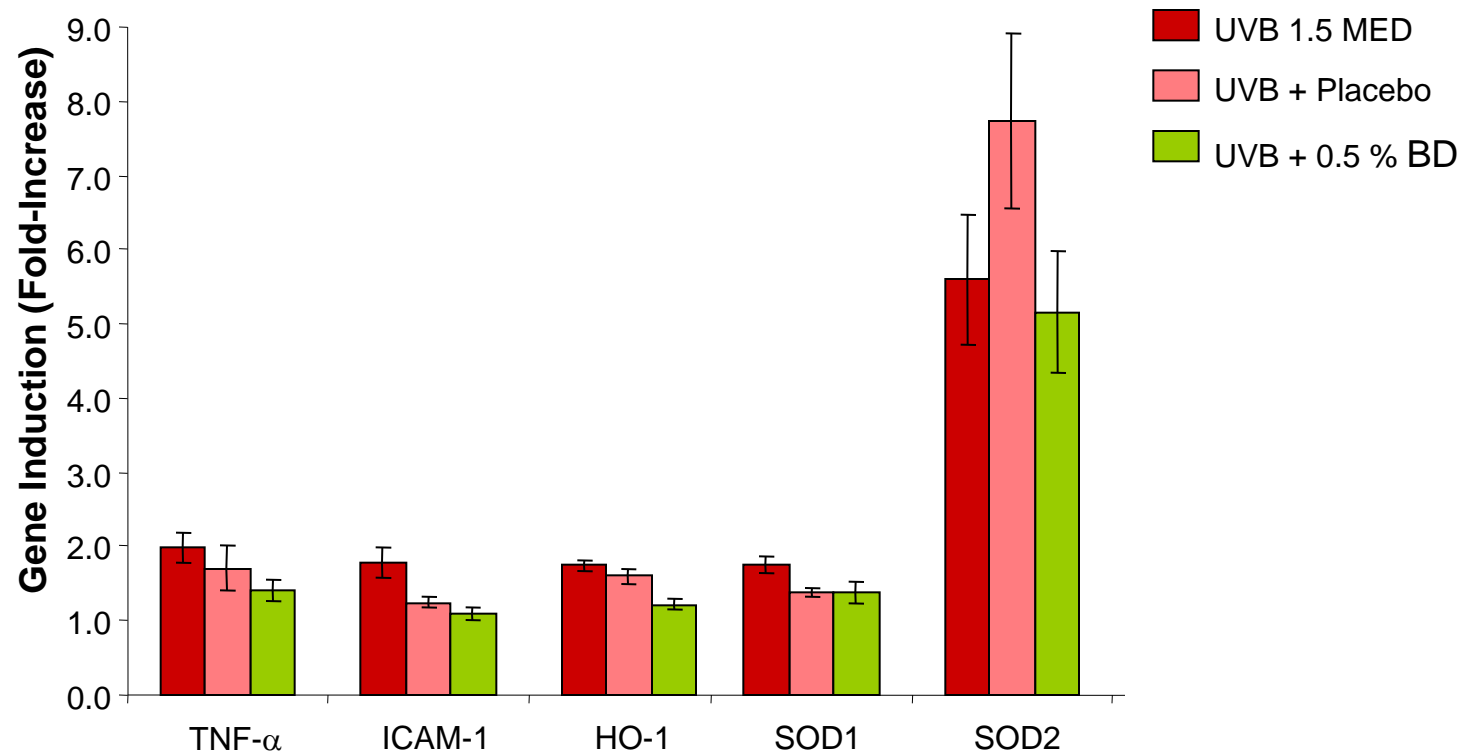
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3. After 24h, 4 mm punch biopsies were taken from the following areas:
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 - UVB-irradiated
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 - Pre-treated with formula containing 0.5 % BDDI, UVB irradiated
4. Preparation and quantification of mRNA for the following markers which are typically induced by UVB:
 - CYP1A1, CYP1A2: foreign chemical and drug metabolism
 - MMP-1: skin aging
 - TNF α , COX-2, ICAM-1 : enzymes involved in inflammation
 - HO-1, CAT, SOD1, SOD2: enzymes involved in oxidative stress

AhR ANTAGONIST

Oxidative and anti-inflammatory enzymes

Results



Topical treatment with BDDI did not have a significant effect on UVB-induced expression of TNF α , ICAM-1, HO-1, SOD-1 and SOD-2 *in vivo*










Safety/Eco Studies




AhR ANTAGONIST

SAFETY/ECO STUDIES

Safety Studies

-  Acute oral toxicity (OECD 423)
 -  Primary skin irritation (OECD 404)
 -  Primary eye irritation (OECD 405)
 -  Lymph node assay (sensitisation OECD 429)
 -  Mutagenicity (Ames OECD 471, mouse lymphoma OECD 476, mouse micronucleus OECD 474)
 -  Skin penetration in-vitro, pig skin (OECD 428)
 -  Repeat dose toxicity & reproductive/development toxicity (28 day OECD 422)
- Acute inhalative toxicity (OECD 403) – Results pending

Ecological Studies

-  Biodegradability (OECD 301F)
-  Acute toxicity to daphnia (semi- static OECD 202)
-  Log Pow (OECD 117)