# LIGHT ACTIVATOR

Superior skin lightening enhancer for a perfect even skin tone





# **TECHNICAL SHEET** Light Activator

**Light Activator** enhances the penetration of Red Light at 633nm (Collagen Light Therapy) and Green Light at 525nm (Whitening Light Therapy) into the skin. Improves skin quality and gives a new vitality to the skin by promoting the production of collagen and elastin while inhibits the production of melanin. It is developed for both the treatments. <u>The new improved formula gives more results in terms of skin brightening and melanin inhibition.</u>

# **ACTIVE INGREDIENTS**

**Arbutin:** more effective, faster, and safer approach to promoting skin lightening and an even skin tone on all skin types. Arbutin blocks epidermal melanin biosynthesis by inhibiting enzymatic oxidation of tyrosine and minimizes liver spots.

**Kombuchka:** with "lipofilling" effect it offers a non-invasive natural way to smoother, younger, more radiant skin; it protects the integrity of collagen and elastin which are essential for maintaining youthful skin.

**Oxi 229-BT:** activates and reactivates the tissutal regeneration connected to the light Therapy through a significant stimulation of cell respiration and has a soothing action on the skin.

**MatrixyI<sup>™</sup>:** a revolutionary ingredient to fight wrinkles, it reduces the appearance of wrinkles. It stimulates fibroblasts to synthesize collagen, elastin, fibronectin and glycosaminoglycans, restoring skin pattern and epidermal-dermal junction to reduce wrinkles.

**Hyalo-oligo**<sup>™</sup>: it covers skin surface avoiding dermal water loss and above all deeply penetrates into the horny layer of your skin, moisturising it from inside and filling wrinkles and lines in a visible way.

**SPF**: guarantee the correct protection of skin.





# Target:

## Ensures an even, lighter skin tone

## Reduces the degree of skin tanning after UV exposure

# Helps to minimize the appearance of spots caused by hyper-pigmentations anomalies:

**1) Melanocytes** proliferate correctly: basically these are pigmented spots caused by temporary or permanent epidermal melanisation unit dysfunction.



**Freckles** are frequently observed in skin areas exposed to the sun (the face, forearms, hands and Legs) in red-haired phototypes. These are eumelanin zones on phaeomelanin backgrounds.



**Chloasma** - pregnancy mask - is a more or less intense, irregular and symmetrical hyperpigmentation Localised on the forehead, around the eyes and the sides of the face. Chloasma is a hyper-secretion of melanin induced by hormonal factors amplified by the effects of the sun.



**Diffuse brown hypermelanosis** is symptomatic of endocrine system disorders or nutritional anomalies.

**Hypermelanosis** can follow cutaneous inflammations. This phenomenon is responsible for the pigmentation of scars and pigmentation caused by irritants combined with sun exposure.

2) Melanocytes\_do not proliferate correctly:



**Lentigines** are small pigmented spots (0.1 to 0.3 cm) which appear on the mid-part of the face. Lentiginosis can be hereditary and is considered to be a disorder which can appear anywhere on the body and persist throughout the winter.



**Solar Lentigo** is a wider lesion than freckles which occurs after serious sunburn on all cutaneous prototypes.



**Senile Lentigo** is generally observed on the back of the hands on older subjects; solar radiation stimulates its development.



#### Post-inflammatory hyper pigmentation (PIH)

Causes skin darkening and discoloration that shows up as spots, or as large patches on a person's body. This happens because of an inflammatory reaction in, or to an injury to, the skin. If the excess melanin is produced in the upper layer of skin (epidermis), the pigmentation colour is a darker shade of brown.



# RESULT

(1)

# Induced a significant reduction in the amount of melanin present both in the skin and on hyperpigmentation spots, study on Asian volunteers [1]

## Significantly smoothes the fine lines of the skin by 16.2%

and increase of luminosity and clarity was perceived for 75% and 81.3% of volunteers: Olive -23% skin tone less dull

Clear pink +20% fresher-looking complexion

With less of the olive and more of the pink colour component, skin has lost some of its tired look and acquires a more glowing complexion [2]





## Skin renewal increase to 25,18% more respect no treated skin.

Activates and reactivates the tissue regeneration through a significant stimulation of cell respiration. [3]



## Stimulation of collagen synthesis increase of 30%

It confirms its strong anti-wrinkle efficacy. [4]

Image analysis by profilometry show:

- Wrinkle depth : 21.6%\*
- Roughness : 16.4%\*
- Wrinkle volume : 24.4%\*
- Main lines density : 46.8%\*
- Wrinkles surface : 67.8%\*
- \*significant with regard to t0 (p<0.01)



**70% of the subjects felt improvement in smooth silky touch** Innovative Hyalo-Oligo that penetrated on the horny layer and, and maintains the skin moisture even after washing [5]

[1] Engelhard, BASF East Asia Regional Headquarters Ltd.

- [2,4] Sederma 29 rue du Chemin Vert 78612 Le Perray-en-Ynes Cedex France
- [3] Pentapharm Ltd, Engelgasse 109, P.O. Box, CH-4002 Basel / Switzerland
  - [5] Q.P.Corporation Fine Chemical Division, Tokyo, Japan

# The quest for a lighter skin tone

In many regions of the world, the concept of beauty is inseparably linked to a light skin tone. The biggest concern for consumers is finding the right product. DSM has addressed this demand with ALPHA-ARBUTIN, which has proven itself the perfect choice for those seeking a lighter, even skin tone. Thanks to its high efficacy, ALPHA-ARBUTIN has gained wide acceptance and is rated highly by people from all walks of life.

Cosmetic products with ALPHA-ARBUTIN typically activate skin lightness by inhibiting tyrosinase, one of the major enzymes involved in the formation of skin tan and age spots. The first visible results can be expected after four weeks, in contrast with other Arbutins like Beta-Arbutin, which take longer to achieve results. *In vivo* studies also demonstrate that ALPHA-ARBUTIN reduces the degree of skin tanning after UV exposure and helps to minimize the appearance of liver spots.

# Key facts

#### Unique product features

- Scientifically proven effects at low concentrations - More effective at 1.0% than Beta-Arbutin *in vivo*
- More effective at 1.0% than Beta-Arbutin *in vivo*
- Nine times more effective than Beta-Arbutin in vitro
- Outstanding tyrosinase inhibition activity in vitro
- Highly pure biosynthetic active ingredient
- High performing enzyme related biotechnology

#### **Benefits**

- Ensures an even, lighter skin tone
- Reduces the degree of skin tanning after UV exposure
- Helps to minimize the appearance of liver spots

#### **Cosmetic application**

- Intensive skin lightening products
- Even skin tone care
- Age spot treatment
- BB/CC creams

### INCI name (active)

Alpha-Arbutin

# Mechanism

ALPHA-ARBUTIN has been specially developed to ensure a lighter skin tone. Unique, high-performing, enzyme-related biotechnology guarantees high purity, addressing increased consumer awareness about safety and efficacy.

Structurally, ALPHA-ARBUTIN (chemical name: 4-hydroxyphenyl- $\alpha$ -D-glucopyranoside) is an  $\alpha$ -glucoside compared to the  $\beta$ -form of the related beta-arbutin.



The a-glucoside bond offers higher efficacy than the  $\beta$ -form. In vitro tests show that ALPHA-ARBUTIN exhibits impressive tyrosinase inhibition and is nine times more effective than Beta-Arbutin. Very low IC50 values (the concentration that produces a 50% inhibition of human tyrosinase) indicates the power of ALPHA-ARBUTIN.

ALPHA-ARBUTIN: IC50 = 1.0 mMol Beta-Arbutin: IC50 = 9.0 mMol

The outstanding efficacy of ALPHA-ARBUTIN is due to its perfect affinity to the active site of tyrosinase. DFT (density functional theory)-optimized structures and ESP (electrostatic potential) calculations on ALPHA-ARBUTIN, Beta-Arbutin, and the substrate of tyrosinase, tyrosine, reveals that the ESP for ALPHA-ARBUTIN is similar to that of tyrosine. The ESP potential for Beta-Arbutin indicates potential difficulties when binding to tyrosinase, meaning the inhibitory activity of Beta-Arbutin is low<sup>1</sup>.





Fig. 1: DFT (density functional theory)-optimized structures and calculated ESP's. The yellow dots represent the negative regions and the blue dots represent the positive regions of the electrostatic potential.

1 Sugimoto Kazuhisa et al., Ezaki Glico Co., Ltd., Biochem. Res. Lab., JPN

# Efficacy

## In vivo

#### One-month skin lightening study

Four emulsions containing ALPHA-ARBUTIN, Kojic acid, Beta-Arbutin and Hydroquinone at 1% use level respectively were applied twice a day for one month on the forearm of 80 women of Chinese descent. The parameter L which represents the lightness of skin was determined by means of chromameter.



Fig. 2: Interaction curve for parameter "L": treated zone vs control, difference M1 = after one month /Mo = start (%)

#### **Results:**

ALPHA-ARBUTIN ensures a lighter skin tone when compared with Beta-Arbutin, Kojic acid and Hydroquinone at 1% use level.

#### Skin tanning reduction after UV exposure

A double blind study was performed on 23 healthy volunteers. The inside of the upper arm of each volunteer was irradiated with ultraviolet rays (1.4 MED) using a solar simulator, and immediately thereafter test samples with ALPHA-ARBUTIN (1% and 2%) were applied twice a day for two consecutive weeks. Skin color ( $\Delta$ L value) was evaluated by means of chromameter one and two weeks after the end of UV exposure.



Fig. 4: ΔL values for ALPHA-ARBUTIN creams one and two weeks after UV exposue

#### **Results:**

ALPHA-ARBUTIN reduces the degree of skin tanning after UV exposure.

#### Liver spot study

A three-month study was performed on 26 women (aged between 40 and 65) with liver spots. Creams including test substances were applied twice daily on different test areas. After three consecutive months of application, the liver spot reduction efficacy was evaluated with the eye using the following five-grade scale: markedly improved, slightly improved, ineffective and aggravated . 53.7% of the panellists reported a markedly improved or improved condition with ALPHA-ARBUTIN against only 30.6% for Beta-Arbutin<sup>2</sup>.



Fig. 3: Satisfaction quotients relating to the evaluation of the liver spot reduction

#### **Results:**

ALPHA-ARBUTIN helps to minimize liver spots.

2 H. Ziegler et al., "The More Effective, Faster and Safer Approach to Skin Lightening and Liver Spot Minimizing," PERSONAL CARE (Jan 2003): 15–18



# Patent N° FR 02.09710 KONBUCHKA<sup>TM</sup>

# Matt complexion **Radiant complexion** Normal surface Irregular surface Good irrigation irrigation

Concept of a radiant complexion

## Function:

Smoothes and freshens the skin

#### **Definition:**

Product of the fermentation of sweet black tea by the symbiosis of two microorganisms

#### **Properties:**

Antiglycation activity, re-densifying effect on the adipocyte population, improves overall skin quality by enhancing skin smoothness, radiance, and color

#### **Characteristics:**

Rich in organic acids and vitamins (group B)

## Points of interest:

Kombucha, also called "long-life fungus", is well known as a beverage in Russia and China and believed to confer longevity

#### **Origin:**

Tradition originated in Russia, Manchuria and Bohemia

#### **INCI name:**

Saccharomyces/Xylinum Black Tea Ferment - Glycerin - Hydroxyethylcellulose

#### **Applications:**

Skin care, particularly anti-ageing or skin smoothing type products





# Kombuchka™

decreases glycation & skin roughness

increases brightness, luster and colour of skin

DO

60

<u>50</u>

40

30 20 23%

. T28

# improves skin radiance

# **CLAIM SUBSTANTIATION**

# In vitro tests

● Anti-glycation power of Kombuchka<sup>™</sup>

Inhibition of glycation reaction with 3% Kombuchka<sup>™</sup>

#### • "Lipofilling" by adipocyte differentiation

Culture of fibroblasts in presence of a differentiation cocktail and 3% Kombuchka<sup>™</sup>. Measurement of G3PDH (*marker for adipocyte maturation from fibroblasts*) activity

## G3PDH activity increase by 136%

# In vivo tests

#### Decrease of skin roughness

14 volunteers. Twice daily application (to the face) of a cream containing 3% Kombuchka<sup>™</sup> against placebo for 29 days. Evaluation with image analysis at D0, D14 and D29

Kombuchka<sup>™</sup> significantly smoothes the microdepressionnary network of the skin by 16.2% after 29 days

#### Improvement of radiance by sensory analysis

16 women. Twice daily application (to the face) of a cream containing 3% Kombuchka™. Evaluation in standardised environment at D0 and D29 by 3 trained experts. Quantified analysis of 3 separate parameters thought to characterize a fresh and radiant complexion: luminosity, clarity and skin colour (validated method, Spincontrol).

79%

	SI	kin colour	
Study evaluating the variations in skin colour using Pantone® type scale of 4 principal colours of the skin			
Colours	Results	Comments	
Red	n.s.	no generation of blotchiness	
Beige	n.s.	no modification of pigmentation	
Olive	-23%	skin tone less dull	
Clear pink	+20%	fresher-looking complexion	

With less of the olive and more of the pink colour component, skin has lost some of its tired look and acquires a more glowing complexion

# **F**ormulation

# Fresh Complexion Cream with Kombuchka™

Part A	%
Water deionized	q.s 100
Ultrez 10 (Carbomer, BF Goodrich)	0.40
Part B	%
Glycerin	5.00
Nipastat (Mixed Parabens)	0.20
Part C	%
Cyclomethicone	3.00
Pemulen TR2 (Acrylates/C 10-30 Alkyl Acrylate, cross polymer, B F Goodrich)	0.20
Part D	%
Crodamol OP (Ethyl hexyl Palmitate, Croda)	4.00
Crodacol CS90 (Cetearyl Alcohol, Croda)	1.00
Crodamol ML (Myristyl Lactate, Croda)	0.30
Crillet 1 (Polysorbate 20, Croda)	1.00
Part E	%
Potassium sorbate	0.10

# In vitro Stimulation of adipocyte differenciation Control Kombuchka<sup>™</sup> 3%





Improvement of luminosity and clarity



The increase of luminosity and clarity was perceived for 75% and 81.3% of volunteers

Tested formulation ref

	SED0207312D
Part F	%
Water deionized	6.00
Sodium Hydroxide 30%	0.60
Part G	%
KOMBUCHKA <sup>™</sup> (Sederma)	3.00
Part H	%
Fragrance	0.10

### Protocol

Part A: Disperse Ultrez 10 into water and let swell for 15 minutes. Melt Part B, add into Part A and heat to 75°C in water-bath. Mix Part D, heat to 75°C in water-bath and pour into Parts (A+B). Then add Part C extemporanouesly. Homogenize well. Add Part E, neutralize with Part F. Homogenize well; then at 35°C add Part G and H.

Non-guarantee: This formulation has been subjected to limited stability tests and has been shown to perform well. However formulators adopting this approach should ensure to their own satisfaction long term stability and functionality. It is good practice to conduct safety tests on all final formulations prior to marketing. Suggested uses should not be taken as an inducement to infringe any existing patents.

# Oxy 229 BT

# For a natural, energetic and renewed skin

What is leading to skin damage?

- Skin is highly exposed to environmental factors like:
- Pollutants, Ozone Damage.
- Diets
- Lifestyle- Stress, fast food, etc.
- and sunlight

..... those lead to damage of skin's cells and create an unhealthy looking skin.



# The solution is Oxy 229 BT

Activity: activates and reactivates the tissue regeneration through a significant stimulation of cell respiration; has a soothing action on the skin

Property	Function	Advantage	
OXY 229-BT is an aqueous solution of a combination of cytoplasmic and mitochondrial constituents (mainly proteins, peptides and amino acids)	Cell respiration: Delivery of energy metabolism stimulating and cell structure forming molecules to the skin cells	The skin is activated and reactivated by OXY 229 BT	
OXY 229-BT is a physiological blend of nutritional molecules	Cell regeneration	OXY 229-BT improves the metabolic situation of "aging skin cells" and increases their regeneration	
OXY 229-BT is a physiological blend of nutritional molecules	Cell turnover	Oxy 229-BT increases skin turnover	
Non animal derived		No BSE risk	
OXY 229-BT is abolutely safe due to its physiological compounds	-		

# Efficacy

## In Vitro: EFFECT OF OXY 229 BT ON FIBROBLAST PROLIFERATION



#### IN VIVO: EFFECT OF OXY 229-BT ON CELL RENEWAL ACTIVITY



□ Racebo ■ 3% OXY 229®-BT ■ 5% OXY 229®-BT

## IN VIVO: EFFECT OF OXY 229 BT ON THE CELL TURNOVER



# **MatrixyI**<sup>™</sup>

# THE MESSENGER PEPTIDE FOR DERMAL MATRIX REPAIR: AN ALTERNATIVE TO RETINOL AND VITAMIN C

#### Function and Characteristics :

MATRIXYL is a hydroglycolic solution containing 100 ppm of lipopeptide Pal-Lys-Thr-Thr-Lys-Ser. This molecule stimulates the skin fibroblasts in order to reconstitute the extracellular matrix: it leads to the synthesis of collagen I and IV, fibronectine and glycosaminoglycans. This physiological activity, demonstrated *in vitro*, is confirmed by *ex vivo* and overall *in vivo* studies.

#### Cosmetic interest (properties):

MATRIXYL constitutes a revolutionary ingredient to treating wrinkles and a remarkable alternative to retinol and vitamin C. Repairing the matrix and the epidermaldermal junction constitutes the mechanism of wrinkle reduction. MATRIXYL also stimulates wound healing gene expression.

#### **Applications:**

Products for wrinkle prevention and repair, eye contour care, mature skin range of products.

Recommended use level: 3 to 8%

CTFA / INCI name:

Glycerin - Water (Aqua) - Butylene Glycol - Carbomer - Polysorbate 20 - Palmitoyl Pentapeptide-3

#### Specifications:

Appearence: opaColour: off-Odour: chapH: 4.0Density  $d_{20}^{20}$ : 1.1Water content (K. Fisher): 20Refractive index (25°C): 1.4Pal-KTTKS content: 90Bacteria: < 1</td>Yeast - Moulds: < 1</td>

Control

Vitamin C

: opalescent gel : off-white : characteristic : 4.0 - 6.0 : 1.140 - 1.160 : 20 - 30% : 1.425 - 1.445 : 90 - 130 ppm : < 100 germs/g : < 10 germs/g

Sederma patent:

FR 2 783 169 EP 99 942 962.4 JP 2000-569 773

WO 00/15188 US 6,620,419

### **CLAIM SUBSTANTIATION**

### IN VITRO

Summary of the results obtained by KATAYAMA (1993) on p - Stimulation of collagen I and III, and fibronectin synthesis	s-Ser (KTTKS): : <b>+ 320%</b> with 50 μM				
Neosynthesis (in these tests, Matrixyl was compared to vitamin C and - Stimulation of collagen IV synthesis on aged fibroblasts - Stimulation of glycosaminoglycan synthesis on fibroblasts	TGFβ):	: <b>+ 257%</b> with 4% MATRIXYL : <b>+ 267%</b> with 2% MATRIXYL			
Molecular biology - Study of gene activation in	Genes	Activity			
tibroblasts and in 3D epidermis:	LOX	collagen cross-linking			
<b>Results:</b> Stimulation of 16 genes specific of skin healing.	MMP3	matrix tissue remodeling			
	FGE response factor	growth factor			
	GSH synthetase	glutathion peroxidase synthesis			
<i>EX VIVO</i> Collagen synthesis rate 250					
- Stimulation of collagen synthesis:					
MATPIXVI 2% (2 ppm pal KTTKS) + 30%	150 -				
	100 -				
MATRIXYL 4% (4 ppm pal-KTTKS) + 117%	50 -				
Vitamin C (1000 ppm) + 42%					

Matrixyl

4%

P 1/3

2%



#### Profilometry and image analysis - 6-month study:

#### Against placebo:

25 volunteers / Twice-daily application on one half of the face a cream containing 3% MATRIXYL versus placebo / Image analysis by profilometry.

- Wrinkle depth - 21.6%\* - Roughness - 16.4%\* - Wrinkle volume - 24.4%\* - Main lines density - 46.8%\* - 67.8%\*
- Wrinkles surface

\*significant with regard to t0 (p<0.01)





#### After treatment



### Against vitamin C:

10 volunteers / Twice-daily application on one half of the face a cream containing 3% MATRIXYL versus a commercially available cream containing 5% vitamin C / Image analysis by profilometry.

	Matrixyl	Vit. C
- Wrinkle depth	- 21.8%*	1.1%ns
- Roughness	- 13.5%**	3.6%ns
- Wrinkle volume	<b>- 24.4%</b> *	0.3%ns
- Main lines density	- 32.8%**	<b>17.8%</b> ns
- Wrinkles surface	- 49.3%**	<b>28.0%</b> ns

\* significant with regard to vitamin C ( $p \le 0.07$ )

\*\* highly significant with regard to vitamin C ( $p \le 0.01$ ) ns no significant

Matrixyl confirms its strong anti-wrinkle efficacy, whereas the cream with vitamin C is inactive.

## Profilometry and image analysis - 2 and 4-month study versus RETINOL:

16 volunteers / daily application for two months then twice-daily application for the following two months / cream containing 3% MATRIXYL versus cream containing 0.07% Retinol / Image analysis by profilometry, dermatological evaluation and echography.



Variation / T0 (% on mean results)



Variation / T0 (% on mean results)

### **Dermatological evaluation**

	MATRIXYL 4%		Retinol	
Variation with regard to T0 (%)	2 months	4 months	2 months	4 months
Forehead	-26.67	-31.11	-20.00	-28.89
Periorbital area	-13.46	-19.23	-11.32	-16.98
Nasogenian groove	-0.20	-20.41	-9.80	-25.49

Echography: improvement of skin thickness







**Before treatment** 

After a 4-month treatment

The anti-wrinkle efficacy of MATRIXYL is confirmed and acts more rapidly than retinol (and without irritation phenomena).

Skin thickness increases by 6.5% in 2 months and 8.6% in 4 months.

Evaluation by the Dermatologist confirms the anti-wrinkle effect, even on the forehead and the perinasal area.

## In vivo study in collaboration with Professor REVUZ from Mondor Hospital

## Profilometry and image analysis - 2 and 4-month study versus placebo

Two groups of 30 volunteers / Twice-daily application on the face and décolleté for 4 months of a cream containing 5% MATRIXYL for one group and a placebo for the other / Image analysis by profilometry on Silflo<sup>®</sup> prints at T0, T2 and T4 months. Biopsies on 12 volunteers.

### **Clinical study:**



## **Biopsies**:

ELASTIN			COLLAGEN IV	
ТО	T4 months	Results	T4 months	Results
MATR		Notable increase of density and thickness of fibres.	MATRIXYL	Regular distribution
Exci	pient	No change	Excipient	Irregular distribution

MATRIXYL, which peptide belongs to the family of Matrikines (Peptidic fragments, messengers of the natural process of tissue repair), is designed to replace retinol and its esters as an efficient anti-wrinkle active ingredient. It acts through specific mechanisms, without any toxicological danger.

MATRIXYL equals and exceeds retinol activity. It can be incorporated in any skin care product, all over the world.

# HYALO-OLIGO High penetration High moisture retentivity

## Features of <Hyalo-Oligo<sup>®</sup>> (The differences from common hyaluronic acid )

<Hyalo-Oligo®>

even after washing.

It is quite a new hyaluronic acid with very low molecular weight (av.7,000 da).

<sup>(1)</sup>Since Hyalo-Oligo<sup>®</sup> covers the skin surface and also

penetrates into horny layer, it keeps the skin

moisture both inner and outer side of the skin. <sup>®</sup>Hyalo-Oligo<sup>®</sup> which remains in horny layer of the

skin is not washed away, thus unlike common

hyaluronic acid, it retains the skin moisture

<Common hyaluronic acid> The skin is coverd by hyaluronic acid as if it is membrane. Л

Loss of skin moisture is prevented.

#### Skin moisture is kept.

Common hyaluronic acid is easily washed away because it doesn't penetrate into the skin

Common Hyaluronic acid





## Skin moisture increase



The gauze soaked in 1% solution of Hyalo-Oligo® was attached to the human skin for 24 hours. (Control 1% solution of common hyaluronic acid ).After the gauze was detached, the skin electric conductivity indicating the amount of skin moisture was measured at three points, namely 1 day after detaching gauze(above 1), 2 days after(above 2), 3 days after (above 3).

## High moisture retentivity

Hyalo-Oligo® keeps skin moisture during 3 days after application. Presented at Annual Meeting of the Pharmaceutical Society of Japan, March 2006 by Takushi Yoshida Q.P.Corporation)



The gauze soaked in 1% solution of Hyalo-Oligo® was attached to the human skin for 8hours x 3days. After this application, the skin electric conductivity indicating the amount of skin moisture was measured at three points, namely1 day after the application (above 1d), 2 days after (above 2 d), 3 days after (above 3 d).

# Efficacy

