M E M O R A N D U M

Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research

Pharmacology / Toxicology Review Memorandum

Date: December 28th, 2007

From: Paul W. Buehler

Through: Abdu Alayash, Basil Golding and Susan Abbondanzo

To: Felice D'Agnillo and Nannette Cagungun

Subject: STN 125267 – C1 Esterase Inhibitor for the treatment of human angioedema (HAE)

Sponsor: Lev Pharmaceuticals Inc.

Receipt Date: August 10th, 2007

Final Review Deadline: December 28th, 2007

Recommendation: STN 125267 is approvable from a pharmacology and toxicology perspective.

Proposed Indication:

C1 Esterase inhibitor is indicated as a C1 Inhibitor replacement therapy for use in patients with Hereditary Angioedema (HAE), also known as C1 inhibitor deficiency. C1 Esterase inhibitor increases antigenic and functional serum levels of C1 inhibitor, thereby replacing the deficient C1 inhibitor activity in pediatric and adult HAE patients to control HAE attacks.

Product Description:

C1 esterase inhibitor packaged in single-use vials that contain 500 Units (U) human C1 inhibitor and is reconstituted with 5 mL of Sterile Water for Injection. Two vials are

combined to provide a single dose of C1 esterase inhibitor at a concentration of 100 U/mL. When reconstituted with the appropriate volume of diluent, contains the following excipients:

Sodium chloride 4.1 mg/ml - per 2 1000 U doses
Sucrose 21 mg/mL - per 2 1000 U doses
Trisodium citrate 2.6 mg/mL - per 2 1000 U doses
L-Valine 2.0 mg/mL - per 2 1000 U doses
L-Alanine 1.2 mg/mL - per 2 1000 U doses
L-Threonine 4.5 mg/mL - per 2 1000 U doses

Roster of Non-clinical Studies:
LEV-NC101 Combined acute and 7-day repeat dose toxicology study with C1-INH in the Sprague-Dawley rat

LEV-NC102 14 day repeat dose toxicology study with C1-INH in the Sprague-Dawley rat

LEV-NC103 Preliminary developmental toxicity study with C1-INH in Sprague-Dawley rats

LEV-NC104 Developmental toxicity study with C1-INH in Sprague-Dawley rats

036-006 In vitro and In vivo (--(b)(4)---- model in rabbits) investigation into the potential thrombogenicity of C1-esteraseremmer-HP

036-007 Pharmcokinetics of C1-esterase inhibitor in rats after transfusion of C1-remmer-HP
036-010 The absence of neo-epitopes in a purified C1-esterase inhibitor preparation (C1-esteraseremmer-HP)

036-010 The absence of neo-epitopes in a purified C1-esterase inhibitor preparation

485936 Single dose pharmacokinetics of two batches of C1-INH after intravenous administration in the male -(b)(4)- rat

Overview of Safety:

LEV-NC101 Combined acute and 7-day repeat dose toxicology study with C1-INH in the Sprague-Dawley rat

Objective: To assess toxicity in Sprague-Dawley rats following single dose intravenous administration of C1-INH for 7-days.

Methods:

Test substance - C1-INH batch --(b)(4)------ (Sanquin)

Vehicle control – Phosphate buffered saline batch ----------(b)(4)-----

Animals – Sprague-Dawley rats (male and female), weight range at start of dosing 124-175 g

Design- acute phase (single dose)

Group	Male	Female	Dose (U/kg)
1	4	4	0
2	4	4	20
3	4	4	100
4	4	4	400

Design-toxicokinetic study (single dose)

Group	Male	Female	Dose (U/kg)
5	6	6	0
6	6	6	20
7	6	6	100
8	6	6	400

Design- Dose ranging (repeat dose)

Group	Male	Female	Dose (U/kg)
9	4	4	0
10	4	4	400
Toxicokinetics (repeat dose)			
11	6	6	0
12	6	6	400

Dosing and route of administration – Animals dosed daily via the tail vein for 7 days with 4.17 mL/kg

Observations:

Clinical – visible signs of reaction to treatment 3 hours post dosing then every 24 hours.

Mortality – checked daily

Body weight – recorded daily
Food consumption – days 1and 8 of repeat dose study

Toxicokinetics:
Blood sampling Day 1 groups 5-8 (10, 30, 90, 180 min, 8 and 24 hours)
Blood sampling Day 1 groups 11-12 (10, 30, 90, 180 min, 8 and 24 hours)

Clinical laboratory studies:
Blood samples obtained at baseline (day 1) and at necropsy (day 8) for hematology and biochemistry.

Terminal observations:
Gross pathology
Organ weights
Histopathology (acute study only)

Results:
Clinical observations -
Phase 1 (acute study-groups 1-4) – no abnormalities at doses from 0-400U/kg
Phase 2 (repeat dose study – group 9 & 10) - no abnormalities at doses from 0-400U/kg

Body weight -
Body weights were not influences by doses up to 400 U/kg in acute or repeat dosing studies

Food consumption –
Food consumption was not influences by doses up to 400 U/kg in acute or repeat dosing studies

Laboratory investigations (hematology) –
Hematology Parameters (Hb, RBC, HCT, MCH, MCHC, platelets, WBC, reticulocytes, neutrophils, lymphocytes, eosinophils, monocytes, basophils, fibrinogen, PT, APTT) were not influences by doses up to 400 U/kg in acute or repeat dosing studies

Laboratory investigations (biochemistry) -
Na, K, Cl, urea, gluc, total protein, albumin, globulins, albumin/globulin ratio, ALP, AST, ALT, Cholesterol, creatinine, Ca, total billirubin, inorganic phosphate and triglycerides were not influences by doses up to 400 U/kg in acute or repeat dosing studies

Organ weights –
Organ weights were not influences by doses up to 400 U/kg in acute or repeat dosing studies

Histopathology findings-
Background abnormalities were noted by the attending pathologist in several animals not specific to any group. There were no statistical trends between group or doses.

Toxicokinetics –
Toxicokinetic analyses were performed in animals treated with doses (20, 100 and 400 U/kg) in the acute phase study and in animals dosed with 400 U/kg in the 7 day study.
Day 1

Acute phase (male rats) Acute phase (female rats)

parameter		20 U/kg	100 U/kg	400 U/kg		20 U/kg	100 U/kg	400 U/kg
t_{last}	hr	24	24	24		24	24	24
AUC_{last}	mU hr/mL	4394	26478	83452		3879	23950	75378
$NAUC_{last}$	mU hr/mL	219.7	264.8	208.6		194.0	239.5	188.4
AUC_{inf}	mU hr/mL	5011	30456	94756		4191	27680	873.11
$NAUC_{inf}$	mU hr/mL	250.5	304.6	236.9		209.6	276.8	218.3
% AUC_{last}- AUC_{inf}	mU hr/mL	0.087	13.06	11.93		7.439	13.48	13.67
λz	1/hr	12.31	0.08420	0.088		0.1062	0.0868	0.0813
t1/2	hr	7.88	8.232	7.866		6.53	7.99	8.53
Cl	mL/h/kg	3.99	3.283	4.221		4.77	3.613	4.581
Vd area	mL/kg	45.37	38.99	47.90		44.93	41.63	56.37
Vd,ss	mL/kg	44.50	38.11	47.33		44.96	42.13	53.16

7 day repeat dose (day 7)

parameter		400 U/kg (male)	400 U/kg (female)
t_{last}	hr	24	24
AUC_{last}	mU hr/mL	61986	79745
$NAUC_{last}$	mU hr/mL	155	199.4
λz	1/hr	0.05367	0.07113
t1/2	hr	12.92	9.745
Cl	mL/h/kg	6.453	5.016
Vd area	mL/kg	120.2	70.52
Vd,ss	mL/kg	112.5	74.60

Reviewer conclusions LEV-NC101: At a maximum dose of 400 U/kg in acute and 7 day dosing in Sprague-Dawley rats no abnormal clinical signs were observed. Unexpected mortalities did not occur in the study. Rat food consumption and body weight remained normal throughout the 7 day treatment period. Intermittent testing of hematological parameter and blood biochemistry remained normal over 7 days of dosing. Tissue gross and histopathology were not different amongst groups based on standard pathology scoring systems. Based on toxicokinetic data there is no apparent cumulative exposure to animals, indicating an adaptive metabolic response to increased levels of C1-INH (provided) data is accurate. The clearance and half-life indicate some degree of plasma accumulation should be occurring after 7 days (i.e. $AUC_{inf(day7)} > AUC_{inf(day1)}$). The Phase III clinical trial (non-prophylaxis) used 20 units/kg as 1-2 doses (approximately 2800 U/treatment day). Therefore, the equivalent highest dose evaluated in this toxicology study without observable adverse events was 400 U/kg x 70kg = 28,000 U (10 fold the maximum clinical dose). The data provided suggests a NOAEL for the study must be set at 400 U/kg since an MTD has not been established in this 7 day toxicology evaluation in rats.

LEV-NC102 14 day repeat dose toxicology study with C1-INH in the Sprague-Dawley rat

Objective: To assess repeat dose toxicity following daily administration of C1-INH to Sprague-Dawley rats for 14-days.

Methods:
Test substance - C1-INH batch ---(b)(4)----- (Sanquin)
Vehicle control – Phosphate buffered saline batch ------(b)(4)-------

Animals – Sprague-Dawley rats (male and female), weight range at start of dosing 131-196 g

Design- Repeat dose study

Group	Male	Female	Dose (U/kg)
1	10	10	0
2	10	10	20
3	10	10	100
4	10	10	400

Design-toxicokinetic study (repeat dose)

Group	Male	Female	Dose (U/kg)
5	18	18	0
6	18	18	20

7	18	18	100
8	18	18	400

Dosing and route of administration – Animals dosed daily via the tail vein for 14 days with 4.17 mL/kg

Observations:
Clinical – visible signs of reaction to treatment 3 hours post dosing then every 24 hours.
Mortality – checked daily
Body weight – recorded daily
Food consumption – days 1and 15

Toxicokinetics:
Blood sampling Day 1 groups 5-8 (10, 30, 90, 180 min, 8 and 24 hours)
Blood sampling Day 14 groups 5-8 (10, 30, 90, 180 min, 8 and 24 hours)

Antibody evaluation:
Blood was drawn for the evaluation of neutralizing antibodies to product and endogenous C1-INH. Baseline anti-C1 was established prior to dosing and then at on day 14 of dosing.

Clinical laboratory studies:
Blood samples obtained at baseline (day 1) and at necropsy (day 14) for hematology and biochemistry.

Urinalysis:
Performed on day 1 and day 14 of repeat dose study

Terminal observations:
Ophthalmology
Gross pathology
Organ weights
Histopathology

Results:
Clinical observations -

Repeat dose study – Groups 1-4 - no abnormalities at doses from 10-400U/kg. One vehicle control group animal died on day 9 dosing due to an injected air bubble otherwise no other clinical abnormalities were reported.

Body weight -
Body weights were not influences by doses up to 400 U/kg in the 14 day repeat dosing study

Food consumption –
Food consumption was not influences by doses up to 400 U/kg in the 14 day repeat dosing study

Ophthalmology –
There were no abnormal opthalmoscopy findings reported in groups 1-4 of the 14 day repeat dosing study.

Laboratory investigations (hematology) –
Hematology Parameters (Hb, RBC, HCT, MCH, MCHC, platelets, reticulocytes, neutrophils, eosinophils, basophils, fibrinogen, PT, APTT) were not influences by doses up to 400 U/kg in acute or repeat dosing studies.

WBC particularly lymphocytes and monocytes were statistically increased compared to vehicle control animals at day 15 in female animals receiving all doses ofC1-INH. This finding was not translated in males and was not dose dependent.

Group	WBC (10x3/uL)	Lymphocytes (10x3/uL)	Monocytes (10x3/uL)	Eosinophils (10x3/uL)
1F	9.3 ± 2.1	7.54 ± 1.69	0.26 ± 0.09	0.16 ± 0.05
2F	12.6 ± 1.9*	10.68 ± 1.72*	0.4 ± 0.16*	0.16 ± 0.05
3F	12.5 ± 2.1*	10.51 ± 1.98*	0.43 ± 0.11*	0.18 ± 0.05
4F	11.1 ± 1.9*	9.18 ± 0.99*	0.33 ± 0.10*	0.21 ± 0.07*

Laboratory investigations (biochemistry) -
Na, K, Cl, urea, gluc, total protein, albumin, globulins, albumin/globulin ratio, ALP, AST, ALT, Cholesterol, creatinine, Ca, total billirubin, inorganic phosphate and triglycerides were not influences by doses up to 400 U/kg in acute or repeat dosing studies

Organ weights –
Organ weights were not influences by doses up to 400 U/kg in repeat dosing studies

Urinalysis-
Urinary parameters were not affected by C1-INH doses up to 400 U/kg

Immunology-
IgG to C1-INH was found to increase in dose dependent fashion. However, since this analysis was only performed on day 14 it is unclear what the time course of antibody generation/neutralization to C1-INH dosing looks like over time and at what point in the study did neutralizing IgG render study results irrelevant.

Histopathology findings-
Background abnormalities were noted by the attending pathologist in several animals not specific to any group. There were no statistical trends between groups or doses.

Reviewer conclusions LEV- 102: At a maximum dose of 400 U/kg in 14 day dosing in Sprague-Dawley rats no abnormal clinical signs were observed. Unexpected mortalities did not occur in the study. Rat food consumption and body weight remained normal throughout the 14 day treatment period. Intermittent testing of hematological parameter and blood biochemistry remained normal over 14 days of dosing. Tissue gross and histopathology were not different amongst groups based on standard pathology scoring systems. Toxicokinetic data on C1-INH at various levels of dosing is of minimal value in this study. First, it is not know how the activity of the C1-INH is affected based a demonstrated antibody response to the product. The assumption would be that product potency is greatly diminished. Thus exposure parameters have little meaning. The equivalent highest dose evaluated in this toxicology study without observable adverse events was 400 U/kg x 70kg = 28,000 U (10 fold the maximum clinical dose). The data provided suggests a NOAEL for the study must be set at 400 U/kg since an MTD has not been established in this 7 day toxicology evaluation in rats. These numbers are of course suspect given the unknown effect of antibodies on toxicology. Nonetheless, antibody generation often becomes a problem with plasma derived human protein in repeat dose animal studies thus the absence of a toxic response at any dose range does not necessarily indicate the product is unsafe. It is this reviewer's opinion that C1-INH, when dosed acutely and repeatedly (at week) intervals is safe based on (1) a lack of toxicity in acute dosing at 10x the maximum clinical dose, (2) a limited potential for accumulation and (3) C1-INH is being used as a replacement with monitoring of plasma levels to ensure appropriate "normal" C1-INH levels are achieved.

LEV-NC103 Preliminary developmental toxicity study with C1-INH in Sprague-Dawley rats

LEV-NC104 Developmental toxicity study with C1-INH in Sprague-Dawley rats

Objective: These two studies were performed with the purpose of establishing a maximum tolerated dose and to establish selection of appropriate lower dose selection in developmental toxicology studies.

Methods:
Test substance - C1-INH batch --(b)(4)------ (Sanquin)
Vehicle control – Phosphate buffered saline batch --------(b)(4)----------

Animals – Pregnant Sprague-Dawley rats ----(b)(4)------ strain

Study design NC103:

Group	Treatment (U/kg/day)	Dose volume (mL/kg)	Animal numbers	
			Main study	toxicokinetics
1	0 (vehicle)	4.17	1-6	25-30
2	20	0.21	7-12	31-36
3	100	1.04	13-18	37-42
4	400	4.17	19-24	43-48

Dosing route/duration – Intravenous via the tail vein for days 6-17 of gestation

Observations:
Clinical – visible signs of reaction to treatment 3 hours post dosing then every 24 hours.
Mortality – checked daily
Body weight – recorded days 4, 6-18 and 20
Food consumption – day 4 – day 20 of gestation

Toxicokinetics:
Blood sampling Day 6 -17 of gestation for main study at time points (10, 30, 90, 180 min, 8 and 24 hours)

Terminal studies (Day 20 of gestation)-
Gross necropsy
Fetal status

Results:

Clinical – There were no clinical observations attributable to C1-INH at any dosing level
Mortality – No unexpected deaths
Body weight –Minor inter-group differences in body weight (not statistically significant)
Food consumption – Minor inter-group differences in body weight (not statistically significant)

Terminal studies (Day 20 of gestation)-
Gross necropsy – There were no reported maternal or embryo-fetal effects of treatment at dose levels up to and including 400 U/kg

Toxicokinetics –
The maximum AUClast values were seen on day 17 (80966 mU.h/mL). After single dosing , the exposure, as reflected by AUClast increased more than dose proportionally. At lower dosing levels 20 and 100 U/kg AUC values were higher after single compared to repeated dosing. Terminal half-life was determined on day 17 to be approximately 8 hours for the 400 U/kg dose.

Reviewer conclusion NC103: There were no maternal or embryo-fetal effects of treatment noted at dose levels up to 400 U/kg/day. It is unknown if there was an antibody mediated neutralizing effect in this study based on NC102 the IgG response was quite profound and did likely influence C1-INHpotential for showing repeat dose toxic effect.

Thus it is not conclusive that repeat dosing of C1-INH is truly non-embryo-fetal toxic. Caution as to use in pregnancy must be noted in the product labeling.

LEV-NC104 Developmental toxicity study with C1-INH in Sprague-Dawley rats

This was a continuation study of LEV-NC104 to increase group size from 6 to 20. The exact same study design and findings were elucidated.

Reviewer conclusion NC104: see comments to NC103

036-006 In vitro and In vivo (---(b)(4)---- model in rabbits) investigation into the potential thrombogenicity of C1-esteraseremmer-HP

Objective: To determine if infusion of C1-INH in an established animal model of thombogenisity will produce blood clots. The in vitro portion of this study involves European Pharmacopoeia standard for evaluation of factor IX containg products (---(b)(4)----------) for the presence of activated vitamin K-dependent coagulation factors (sensitivity of the test system to IXa > Xa > thrombin > VIIa).

Methods: See the European Pharmacopoeia for --(b)(4)-- testing methods (in vitro). The ---(b)(4)--- model is the established in vivo model for evaluation of clot formation in vivo. The extent of thrombogenic effect following injection of product into the ear vein is determined by the scoring system defined as 0 = no clots, 1 = macroscopic fibrin threads, 2 = small clots, 3 = two or more larger clots, 4 = one clot equaling the size of the tied off segment.

Results:

In vitro (-(b)(4)- test) C1-INH

Test	Claim	C1-INH	Heat treated C1-INH
-(b)(4)-			
1/10 dilution (sec)	>150	251 ± 14	336 ± 43
1/100 dilution (sec)	>150	266 ± 7	304 ± 56
Blank (sec)	>200	283± 7	341 ± 24
Thrombin test			
6 hours 37 C	Negative	Negative (3/3)	Negative (4/4)
24 hours ambient	Negative	Negative (3/3)	Negative (4/4)
Chromegenic substrates (1000xΔA405/min)			
APC,K/Pl	No claim	0.1 ± 0.2	0.0
K, Pl,Xa/IIa	No claim	0.0	0.4 ± 0.2
Xa,XIIa	No claim	0.3 ± 0.5	0.5 ± 0.3
IIa	No claim	0.6 ± 0.1	0.6 ± 0.4

Based on these results the product does not contain K^+ dependent coagulation factors

In vivo (-(b)(4)- model) C1-INH

Dose (U/kg)	N	N ≥ scores of 1 (total score)	Max. score	Index Total/max
20-50	4	1	16	0.06

50-100	9	7	36	0.19
100-200	5	3	20	0.15
>200	6	12	24	0.50

In vivo (-(b)(4)- model) C1-INH (heat treated)

Dose (U/kg)	N	N ≥ scores of 1 (total score)	Max. score	Index Total/max
50-100	2	0	8	0.00
100-200	3	2	12	0.42

Reviewer conclusion 036-006: Data presented from the ---(b)(4)----- model demonstrates that C1-INH presents a thrombogenic risk defined by a threshold between 100-200 U/kg. Lower doses are not without risk however a minimum potential for clot formation is demonstrated at 20-50 U/kg. As a result the definition of a NOAEL for this product should not be defined above 50 U/kg. Exceeding recommended doses of C1-INH (> 20 U/kg max of two doses) can have thrombogenic risk.

036-007 Pharmcokinetics of C1-esterase inhibitor in rats after transfusion of C1-remmer-HP
036-010 The absence of neo-epitopes in a purified C1-esterase inhibitor preparation (C1-esteraseremmer-HP)

Reviewer conclusion 036-007: This study provides no relevant information toward the approval of this product.

036-010 The absence of neo-epitopes in a purified C1-esterase inhibitor preparation

Objective: The 10 hour 60 °C pasteurization of the current product may cause subtle changes in the spatial structure of the molecule (neo-epitope) which may elicit an immune response when infused. Presently, the pasteurization process has not caused changes in potency. The purpose of this study was to determine if pasteurization C1-INH causes neo-epitope formation.

Methods: Rabbits were dosed via the intravenous (6 weeks) or intramuscular (12 weeks) routes. Two sampling times were designated other than baseline and terminal sampling. The

designated sampling points were two and four weeks for the intravenous dosed animals and three and six weeks for the intramuscular dosed animals. Complement inactivation and antibody formation (---(b)(4)---- method) was determined in serum.

Results: No cross reacting antibodies were detected in baseline serum (rabbits have no natural cross-reactivity to human C1-INH). Antibody production was found to be faster in intravenous dosed animals. No neo-epitopes generated antibodies were observed in intravenous or intramuscular dose inoculated animals. Thus there were no differences detected in heat treated versus non-heat treated C1-INH.

Reviewer conclusion 036-010: There is minimal risk of neo-epitope induced antibody formation as a result of heat treating C1-INH for 10 hr at 60 °C.

485936 Single dose pharmacokinetics of two batches of C1-INH after intravenous administration in the male -(b)(4)- rat

Objective: Demonstrate the pharmacokinetic bioequivalence based on exposure parameters of two lots of C1-INH that have been produced via differing production processes at different manufacturing sites.

Methods: Two dosing levels (20 and 400 U/kg) were evaluated in single dosed male (b)(4) rats.

Study design

Period	Group	Product	Dose (U/kg)	Dose (mL/kg)	Number of animals	Animal numbers
1	1	178479/A	20	0.194	6	1-6
1	2	178479/A	400	3.88	6	7-12
1	3	178479/B	20	0.179	6	13-18
1	4	178479/B	400	3.57	6	19-24
2	1	178479/B	20	0.179	6	1-6
2	2	178479/B	400	3.57	6	7-12
2	3	178479/A	20	0.194	6	13-18
2	4	178479/A	400	3.88	6	19-24

Results: Plasma concentration versus time curves of the test compound from both manufacturing processes was similar. AUC_{last} values were obtained on day four post dosing making $AUC_{0\text{-}inf}$ approximately 15% extrapolated. In this study dosing of revised

manufacturing process C1-INH at 20 U/kg resulted in AUC_{last} values being 97% of the original manufacturing process. At doses of 400 U/kg AUC_{last} values were 86% of the original manufacturing process. The accurate determination of bioequivalence in rats based on $AUC_{0\text{-}inf}$ was not accurate based on greater than 10% extrapolated values.

Reviewer conclusion 485936: The new and original manufacturing process end products a equivalent based on pharmacokinetic comparisons in the rat. The potency and or potential toxicity can not be commented on since these were not an end point of the study.

Final end product contents:

Assay	Unit	Requirement
C1-inhibitor	U/ml	[redacted]
Protein	g/l	
Specific C1-inhibitor activity	U/mg	
Sterility		sterile
(b) (4)		[redacted]
pH (directly measured in solution)		6.6 - 7.4
(b) (4)	(b) (4)	[redacted]
[redacted]		
Stability (after dissolving 3 h at RT)		
(b) (4)	[redacted]	
Sodium	mmol/l	
Sucrose	mmol/l	
Citrate	mmol/l	
Valine	mmol/l	
Alanine	mmol/l	
Threonine	mmol/l	
(b) (4)	(b) (4)	
Tests not required for release of the product		
[redacted]	(b) (4)	

Protein	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)		(b) (4)

Impurity testing has not been performed with C1-INH. However, the proposed specifications for process related impurities are not in excess compared to other human plasma derived products and there were no overt toxicity issues in 7 and 14 day repeat dose toxicology studies which exposed animals to 10x the maximum clinical dose of C1-INH end product.

Reviewer recommended product labeling changes:

USE IN SPECIFIC POPULATIONS

(b) (5)

--(b) (5)--

13.1 ---

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13.2 --

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