IN VITRO AND *IN VIVO* KILLING EFFICIENCY OF ELIMAX

(1) a. *in vitro*

Lice (10-15) were placed on small sieves and 2ml of test product was dripped over the lice. The product was allowed to drain through the sieve and the lice were left undisturbed for several minutes (timing to be chosen). After this the lice were only rinsed with tap water (150ml) until all product had visually disappeared through the sieve. The sieve was then placed on a paper towelette to drain the remaining water of the sieve and subsequently placed in a fresh petri dish. The viability of the lice was monitored after 5, 15, 30, 60, 120 minutes using a binocular and recorded.

Product ref.	Product	Date	# lice	5'	15'	30'	60'	120'	% killed
X92001483	Elimax shampoo	4/11/2013	10	9	10	10	10	10	98%
X92001483	Elimax shampoo	9/12/2013	30	30	30	29	30	30	99%
X92001483	Elimax shampoo	03/02/14	9	9	9	9	9	9	100%
X92001483	Elimax shampoo	11/03/14	10	10	9	9	9	9	92%
								average	**97%***

*This value is not significantly different from 100% (One-sample t-test, p=0.99)

(1) b. *in vivo:* clinical trial

Name of Sponsor:	OYSTERSHELL, NV
Name of Finished Product:	X92001327, Elimax lotion
Active Ingredient:	Works by suffocation
Title of Study:	A Randomized, Controlled, Investigator-Blinded, Comparative Study to Evaluate the Safety and Efficacy of product X92001327 versus RID Shampoo in Subjects with Head Lice
Investigator:	Lidia Serrano, Michelle Gonzalez, MD
Study Centers:	1
Publication:	No publication at the time of the report
Studied Period:	05 March 2013 to 20 June 2013
Date or Report	12th of August 2013
Objectives:	The primary objective of the study was to compare the safety and efficacy of X92001327 versus RID in subjects with head lice. The subjects received a single application on Day 0 of either X92001327 or RID shampoo based on the randomization schedule. A repeat application of the test product was administered on Day 7. Subjects visited the clinic four times: on Day 0, Day 1, Day 7 and Day 10.
Number of Patients:	**Planned:** 50 **Enrolled:** 60 **Analyzed:** 50 (efficacy); 60 (safety)
Diagnosis and main inclusion criteria:	Head Lice

	Male or female over the age of 1 with active head lice infestation of live lice at the screening visit and a presence of nits who agree to not use any other pediculicides or medicated hair grooming products for the duration of the study.
Test Product, dose, and mode of administration,	Saturate hair with X92001327 product and keep on the hair for 15 minutes; then add a good measure of regular shampoo. Work the shampoo into the treated hair; add a little water to work up a lather and rinse out. Repeat shampooing if necessary. Towel dry after rinse-out. The hair was then combed with a nit comb.
Lot Number:	20121119
Expiration Date:	10/2014
Duration of Treatment:	1 application on each of Days 0 and Day 7
Reference:	RID shampoo
Therapy, dose and mode of administration	Saturate hair with RID shampoo and keep on for 10 minutes; rinse with warm water and then towel dry. The hair was then combed with a nit comb.
Batch number:	Lot Numbers: 5402HW6, 5402H7C, 5402GCL
Expiration Dates:	8/2014, 6/2014
Criteria for Evaluation:	Count of Live Lice present
Statistical Methods:	Efficacy analyses (intention to treat analyses) included all patients who received two treatments. Subjects who had received at least one treatment and had at least one safety assessment after treatment were included in the safety analysis. For the primary endpoint, the odds ratio with two-sided 95% confidence interval was calculated to compare the cure rates of the two treatments at the end of the study. The null hypotheses that the odds ratio equal to 1 was tested with the Fisher's Exact test at α-level of 0.05. The proportion of subjects free of lice in each treatment group post-dosing (Day 1), and at the Day 7 and Day 10 visits was calculated. Next, the efficacy at each time point was compared by calculating the common odds ratio of the cure rates for both treatments. Under the null hypothesis that this odds ratio equals 1, this was tested with the Cochran-Mantel-Haenszel test for conditional independence. Descriptive statistics of all demographic, baseline variables and study parameters were provided overall. Continuous data were summarized by their mean, standard deviation, median, minimum and maximum. Categorical and ordinal data were summarized by frequency and percentages.
Summary Results:	**Demographics and infestation grade** The mean age of the subjects was 15.7 years with 93.3% of the subjects being female. The distribution of age, gender, race, ethnicity and size of the household was more or less similar for both treatment groups. Hair characteristics differed somewhat with the X92001327 group having on average longer hair and more straight hair than the RID shampoo group (shorter and curlier hair). At inclusion, the number of viable eggs ranged from 4 to 280 (median 34.5) and from 8 to 235 (median 41) in the X92001327 and RID group, respectively. The number of live lice (adults and nymphs) at day 0 ranged from 5 to 95 (median 15.5) and from 5 to 109 (median 23.5) in the X92001327 and RID group, respectively. In practice, people with at least 25 lice of all stages are defined as having heavy infestation (Burgess *et al.*, 2010), which means several included subjects were heavily infested with lice. **Efficacy Results:** The proportion of lice free subjects for the X92001327 treatment on Day 1, Day7 before treatment, D7 after treatment and on day 10 were 56.7% (17/30), 56.7% (17/30), 83.3% (25/30), and 80% (24/30), respectively. For the RID shampoo group this was 55% (11/20), 30% (6/20), 85% (17/20), and 45% (9/20), respectively. Out of the six failures from the

	X92001327 group, four subjects were identified as having a heavy infestation at the start of the study (between 37 - 95 lice). Out of the 11 failures from the RID group, five subjects had a heavy infestation at the start of the study (between 26- 42 lice). The ratio of the cure rate at each time point was compared by calculating the common odds ratio. The common odds ratio was 1.74 (95% CI: 0.86; 3.56, p=0.174). The efficacy of X92001327 versus RID shampoo on Day 10 showed that treatment with X92001327 resulted in a statistical significant improvement of lice free subjects compared to RID shampoo. The odds ratio on Day 10 was 4.71 (95% CI: 1.19; 20.9, p=0.015), subjects treated with product X92001327 were 4.71 times more likely to be lice free by Day 10 then subjects from the RID group. The cure rate of X92001327 was 80% on Day 10 whereas the cure rate for RID shampoo was 45%. Since several subjects suffered from a heavy infestation (≥25 lice, cfr. Burgess et al., 2010), the cure rate was also calculated by infestation grade: a light to moderate group with less than 25 lice and a heavy infestation group with subjects having 25 lice or more. The efficacy of X92001327 was related with the infestation degree, a light to moderate infestation resulted in a lice free rate of **94.1%** whereas the cure rate of heavy cases was only 61.5%. For RID shampoo no relation was observed between the infestation degree and the cure rate which was 40 and 50% for light to moderate and heavy infestation, respectively. **Safety Results** The majority of the subjects suffered from pruritus (mild to severe) at the start of the study. Pruritus decreased during the course of the study and by Day 10, 50% (15/30) of the subjects treated with X92001327 and 60% (12/20) of the subjects treated with RID shampoo did not complain about pruritus. For X92001327 this was related to successful treatment, 14/15 subjects without signs of pruritus were lice free. For RID shampoo only 6/12 subjects that did not complain about pruritus were lice free. None of the subjects suffered from infection during the observation period. Excoriation and dry scalp was observed for a few subjects only. Excoriation was related with the condition before the start of the study (pre-treatment Day 0). Furthermore, the eyes of all subjects were clear at all observation points and for both treatments. Subjects who had received at least one treatment and had at least one safety assessment after treatment were included in the safety analysis. This meant all enrolled subjects (60), 39 subjects received treatment with product X92001327 and 21 subjects received treatment with RID shampoo. Adverse events were only recorded for X92001327 subjects and were limited to transient mild to moderate erythema of the neck and/or shoulder region.
Conclusions:	The efficacy and safety of X92001327 versus RID shampoo in subjects with head lice was assessed. The cure rate on day 10 for X92001327 was 80% versus 45% for RID shampoo. Treatment with X92001327 resulted in a statistical significant higher proportion of lice free subjects compared to RID shampoo. Subjects treated with X92001327 were 4.71 times more likely to be lice free by day 10 then subjects from the RID group. When the degree of infestation at the start of the study was taken into account, a cure rate of **94.1%**, and 61.5% was obtained for X92001327 for subjects with a light to moderate (< 25 lice), and heavy (≥25 –lice) infestation, respectively. For RID shampoo no relation was observed between the degree of infestation.