This poster presents nonclinical research conducted to characterize mechanisms underlying findings observed in rat pre- and postnatal development studies for scientific exchange. The data presented do not change the approved indication, contraindications, or prescribing recommendations for oteseconazole. Healthcare professionals should refer to the FDA approved Prescribing Information for complete safety information.

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Dietary Vitamin K–Dependent Coagulopathy and Ocular Bleeding Events in Rat Pups Treated With Oteseconazole During Peri-Postnatal Development (PPND) Studies

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Background

Oteseconazole (VIVJOA®) is an antifungal agent approved for recurrent vulvovaginal candidiasis (RVVC), however its use is restricted to women not of child-bearing potential. This restriction is based on rat PPND studies describing a higher incidence rate of ocular opacities and ocular and systemic bleeding events in treated pups compared to untreated controls. Ocular opacity incidence rates historically range from 0-50% in young rats (Ban et al., 2008). Investigations were initiated to better define the translational relevance of PPND findings and gain mechanistic understanding of treatment-related coagulopathy.

Methods

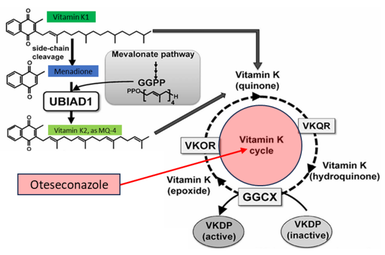
Initial PPND studies used pregnant Sprague-Dawley Crl:CD(SD), Hsd(SD), or Crl:WI(Han) dams dosed with oteseconazole (OTE) (7.5 mg/kg/d) from gestation day 6 (GD6) through lactation day 20 (LD20) that included full ophthalmic and gross necropsy examinations performed on PND21/35. Clinical pathologic testing included blood cell counts, coagulation screening tests (aPTT/PT), and serum chemistry panels. Because subsequent PPND and cross-fostering studies demonstrated variability in spontaneous background and treatment-associated ocular abnormalities between contract research sites, potentially related to feed composition (PMI 5002 vs Teklad 2016C), assays to detect vitamin K deficiency were performed. These assays included measurement of plasma vitamin K and procoagulant activities of vitamin K-dependent factors (II, VII, IX, X). In addition, a subset of rats was administered intramuscular vitamin K daily (10 mg/kg phylloquinone). Subsequent PPND studies evaluated diets with varying vitamin K content (2016C>5002>custom blend).

	ANALYSIS OF DIETS USED				
	LABCORP STUDY NO. 8525-516		CRL STUDY NO. 32011898		
	PMI 5002	2016C	PMI 5002	2016C	Custom Blend
Vitamin K1, ppm	0.335	0.035	0.448	0.062	0.138
Vitamin K3, ppm	0.071	2.322	0.047	2.282	<0.009
Total Vitamin K Equivalents, ppm	0.406	2.355	0.495	2.344	0.140

- Analytical sensitivity issues precluded determination of vitamin K-epoxide formation, therefore the inhibitory potential of oteseconazole was measured in rat and human microsomal vitamin K-epoxide reductase (VKORC1) prepared from transfected yeast cells, using warfarin as a known inhibitor. Human plasma samples from previous clinical trials were obtained with IRB approvals.
- VKORC1 protein was expressed in Pichia pastoris yeasts. Yeasts were harvested by centrifugation. Microsomes were isolated by homogenizing yests with zircon beads in phosphate buffer (pH 7.4) and centrifuged. Pellets were crushed in HEPES buffer with 20% glycerol.
- Enzymatic assays were conducted with vitamin K1 epoxide as substrate and either oteseconazole or warfarin as inhibitors. Incubations at 37°C were stopped after 30 minutes with the addition of isopropanol. Vitamin K1 (phylloquinone) was solubilized by addition of hexane followed by evaporation and resuspension in methanol. Vitamin K1 concentrations were determined by LC/MS.

Vitamin K cycle: VKOR enzymes convert inactive vitamin K (epoxide form) to active vitamin K (quinone form), which is essential for the activation of coagulation factors.

The site of oteseconazole inhibition of the VKOR cycle is unknown.



Disclosures: These work were sponsored by Mycovia Pharmaceuticals Inc. (Mycovia), the manufacturer of oteseconazole

Results

PPND clinicopathologic findings in pups included subcutaneous and ocular hemorrhage, early mortality, pronounced prolongation of aPTT/PT, and decreased fibrinogen and red cell counts (Cmax>40μM). The presence and severity of signs appeared variable between sites that fed either 5002 or 2016C diets as their standard rodent chow. Studies revealed reductions in maternal and/or pup plasma, liver and ocular vitamin K and low activities of factors II, VII, IX, X in pups. Marked prolongation of aPTT/PT after 28 days of oteseconazole dosing (15 mg/kg/d) in young adult rats fed 5002 diet were reversed by daily phylloquinone administration (10 mg/kg i.m.) through day 35.

Summary of aPTT (clotting time in seconds; mean with SD) By Timepoint/Treatment and Group/Sex

Timepoint	Ctrl-Low VitK-Male	OTE-Low VitK-Male	Ctrl-Low VitK- Female	OTE-Low VitK-Female
Predose	13.9 (1.0)	13.7 (1.6)	13.5 (1.4)	13.9 (3.7)
Day 28	17.4 (2.0)	40.8 (31.6)	13.9 (4.9)	21.7 (2.6)
Day 35	13.2 (3.5)	13.0 (1.9)	14.3 (1.2)	11.7 (1.2)

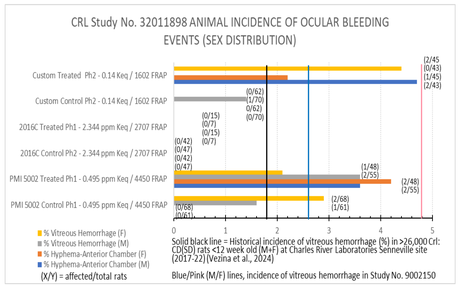
Control vs. OTE treated: p<0.05 (M/F), OTE treated Day 28 vs. Predose: p<0.05 (M/F), OTE treated Day 35 vs. Day 28: p<0.05 (M/F)

Summary of PT (clotting time in seconds; mean with SD) By Timepoint/Treatment and Group/Sex

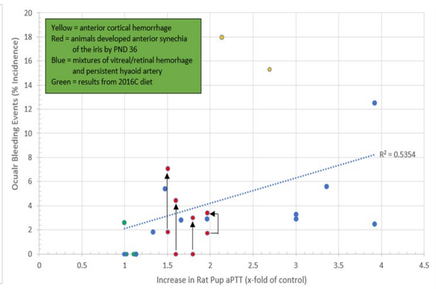
Timepoint	Ctrl-Low VitK-Male	OTE-Low VitK-Male	Ctrl-Low VitK- Female	OTE-Low VitK-Female
Predose	19.5 (1.0)	20.6 (0.9)	20.8 (1.0)	19.9 (0.6)
Day 28	21.8 (0.4)	37.9 (29.6)	21.4 (0.8)	18.9 (3.2)
Day 35	25.2 (0.9)	23.5 (1.1)	22.7 (1.0)	21.5 (0.6)

Control vs. OTE treated: p<0.05 (M), OTE treated Day 28 vs. Predose: p<0.05 (M), OTE treated Day 35 vs. Day 28: p<0.05 (M)

Coagulopathy with ocular bleeding was attenuated/absent in pups from maternal rats fed the 2016C diet but present for diets with lower vitamin K (5002 and custom).



Study No. 32011898 Animal Incidence of Ocular Bleeding Events (Sex Distribution)



Correlation of Systemic Coagulopathy to Ocular Bleeding Events (PND17-30) in Rat Pups from Treated Dams

Rat VKORC1Inhibition by Oteseconazole

IC50 (μM)		73.90 ± 13.23	
	Exp1	Exp2	Exp3
IC50	87.13	73.92	60.66
r^2	0.9742	0.9754	0.9657

Rat VKORC1Inhibition by Warfarin

IC50 (μM)		0.14 ± 0.01	
	Exp1	Exp2	Exp3
IC50	0.15	0.14	0.13
r^2	0.9810	0.9917	0.9857

Human VKORC1Inhibition by Oteseconazole

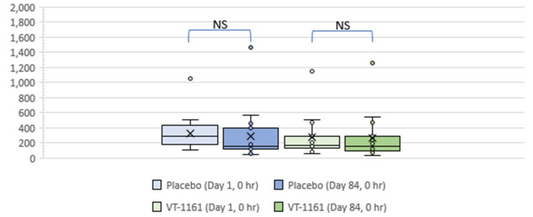
IC50 (μM)		53.40 ± 7.73	
	Exp1	Exp2	Exp3
IC50	54.19	60.70	45.30
r^2	0.9779	0.9601	0.9474

Human VKORC1Inhibition by Warfarin

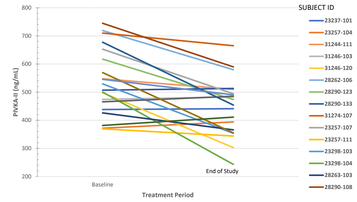
IC50 (μM)		0.56 ± 0.04	
	Exp1	Exp2	Exp3
IC50	0.61	0.55	0.53
r^2	0.9605	0.9348	0.9638

Results

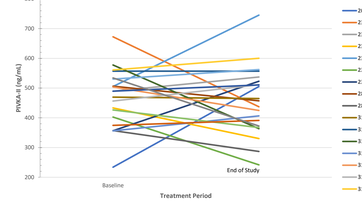
Oteseconazole inhibition of rVKORC1 and hVKORC1 showed IC50 values of 74 and 53 μM compared to 0.14 and 0.56 μM for warfarin, respectively. Systemic exposure margins of 0.8x the IC50 for rVKORC1 on PND10 lead to coagulopathy and ocular bleeding in rat pups on the 5002 and custom diet while systemic/liver exposure margins of 0.42/9.8x the IC50 for rVKORC1 inhibition on GD18 led to aPTT increases in pregnant rat dams on the custom diet. In humans, aPTT/PT were unchanged in Phase 1 single and multiple ascending dose trials, plasma vitamin K was not altered and protein induced by vitamin K absence or antagonist-II (PIVKA-II) was unchanged in clinical trial subjects after 12-week of treatment in Phase 3, randomized, double-blind, placebo-controlled studies where Cmax=5.3μM.



Plasma K1/Phylloquinone Concentrations (pg/mL) in RVVC Clinical Trial Subjects Pre-Dose and After a 12-Week Treatment with VIVJOA Compared to Placebo (CL-012)



VMT-VT-1161-CL-012 Clinical Study Results for PIVKA-II (Placebo Group)



VMT-VT-1161-CL-012 Clinical Study Results for PIVKA-II (VIVJOA Group)

Conclusions

- While choice of rat strain and selected CRO facility influenced study outcomes, wide variability in vitamin K content across diet selection (natural phylloquinone or added menaquinone) had direct effects on coagulopathy and could account for variable incidence of ocular bleeding events in PPND studies.
- Oteseconazole was determined to be a weak rat VKORC1 and weaker human VKORC1 inhibitor, at least 100x less potent than warfarin.
- The absence of animal diet content monitoring, and the limited regulatory oversight can significantly influence drug development.
- Inhibition of VKORC1 in rat is observed at oteseconazole concentrations similar to the rat pup exposures in PPND studies and could explain the coagulopathy-associated effects in treated rat pups.
- Weak hVKORC1 enzyme inhibition results in a therapeutic exposure margin of at least 10x, suggesting limited relevance to humans.
- No reports of coagulopathy-type findings in humans to date.

References

[1]Ban Y, Tomohiro M, Inagaki S, et al. Spontaneous ocular abnormalities in Crl:CD(SD) rats. Anim Eye Res. 2008;(29)9-15.

Acknowledgments

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