Clinical evidence of hepatoselectivity of oral pentamidine (VLX103) in cirrhotic patients with early stage hepatocellular carcinoma

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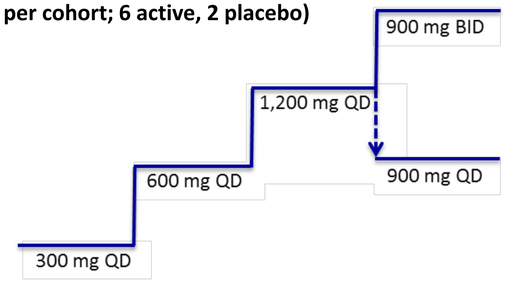
1. ABSTRACT

VLX103 is the first oral pentamidine isethionate (PI) formulation ever administered to humans. Animal pharmacological studies have recently shown that PI exerts significant anti-inflammatory and hepatoprotective effects through lipopolysaccharide (LPS) neutralization and prevention of the TLR4 mediated signaling pathways. Hepatoselectivity of PI is a desirable feature in the treatment of chronic liver diseases in order to spare the organs which are usually the target for PI toxicity (kidneys and the pancreas). Since hepatoselectivity has already been shown to be achievable following oral administration in a cross species animal model, clinical confirmation of this unique profile was sought in a Phase I study conducted in patients with liver disease. **Methods**: Four ascending doses of VLX103 or a matching placebo (300, 600, 900 and 1,200 mg) were sequentially administered daily over three days to different cohorts (N:8) of adult, early stage HCC and Child-Pugh A or B cirrhotic patients. Twenty four hours post treatment, all subjects underwent thermal ablation of their hepatic tumor, which allowed sampling of tumoral and non-tumoral hepatic tissue through needle biopsy. Tissular and plasma PI was assessed using a sensitive LC-MS/MS method. **Results**: A total of 28 patients were randomized in this study. All dosing cohorts were completed except at 1,200 mg QD because of GI intolerance. At the 300, 600 and 900 mg QD doses, plasma pentamidine concentrations were very low or below the level of quantification (BLQ = 2.5 ng/mL). Consistently quantifiable plasma drug levels were only found at the 1,200 mg dose. As anticipated however, significant hepatic concentrations were found in both tumoral and non-tumoral tissue at all doses, with a linear dose-concentration relationship (median of 13 to 147µM in non-tumor, 10 to 56µM in tumor). These concentrations are therapeutically relevant, since they correspond to a multiple of preclinical IC50 data, and in line with those predicted by a cross-species PK predictive model. Using the single highest recorded oral plasma Cmax of 0.06µM at the oral dose of 600 mg (the anticipated maximum daily dose), and the mean tissue concentration, 24-hour post last dose, of 31µM (n=6) in the non-tumoral biopsy samples, we calculate an underestimated tissue/plasma ratio of about 4,000 to 16,000. **Conclusions**: These findings confirm the hepatoselectivity of oral VLX103 in liver disease patients, even more selective than reported for IV administered pentamidine. This is most likely attributable to a high distribution volume of pentamidine in the liver and the rapid hepatic first pass uptake following oral administration.

2. STUDY DESIGN

Figure 1. Phase 1 Study Design: Randomized, double-blind, placebo controlled, dose ascending with distinct patients cohorts (n=8 per cohort; 6 active, 2 placebo)



Study Patient Population

- Male or female subjects, 18 years of age or older
- HCC with a Barcelona score of 0 or A, tumor diameter ≤ 5 cm
- Suitable for and scheduled to undergo thermal ablation
- Child Pugh score of A or B cirrhosis

Primary Endpoint

Liver concentration of pentamidine in hepatocellular carcinoma tumor and surrounding tissue after oral administration for 3 days at different doses, measured in liver tissue obtained during thermal ablation procedure or after partial hepatectomy.

Biopsies and liver tissue sampling

Two biopsy samples were obtained prior to thermal ablation: one from the liver and one from the tumour in addition to the one(s) usually performed. Tissue samples were inserted in labeled culture tubes which were placed on dry ice for 15 minutes to be rapidly frozen.

Bioanalytical Method

Pentamidine was assayed in plasma and tissue samples using a validated Liquid Chromatography method coupled to a tandem Mass Spectrometry (LC-MS/MS) detection system.

3. DEMOGRAPHICS

Table 1. Patient Demographics

Gender	**97 % male**
Race	79 % Caucasian
HCC stage	75 % Barcelona 0
Cirrhosis stage	75 % Child-Pugh A

4. RESULTS

Pharmacokinetics and Hepatoselectivity Results

At the 300, 600 and 900 mg QD doses, mean and median plasma pentamidine concentrations were very low or even below the level of quantification (BLQ). Significant plasma drug levels were reported only at the 1,200mg dose. However, significant hepatic tissue concentrations were found in both the tumoral and non tumoral tissue at all doses, with an apparent linear dose-concentration relationship. Moreover, the hepatic drug levels were similar to those predicted by Verlyx's animal cross-species PK predictive model. These findings truly confirm the hepatoselectivity of VLX103.

Three consecutive days of daily VLX103 treatment led to therapeutically relevant hepatic concentrations, corresponding to a multiple of previously reported preclinical IC50 data. These hepatic levels are expected to exert the pharmacological and therapeutic effects required to reduce inflammation, hepatocyte damage, steatosis and fibrosis.

Table 2. Pentamidine Plasma and Liver Tissue Pharmacokinetics

Dose	Plasma C_{max} (Day 3 Range)	Median Tumor Conc.	Median Non Tumor Liver Conc.	Model Predicted Liver Conc.
300 mg	BLQ-0.03 µM	10 µM (n=5)	13 µM (n=5)	15.3 µM
600 mg	BLQ-0.06 µM	18 µM (n=5)	33 µM (n=5)	30.6 µM
900 mg	BLQ-0.09 µM	7.4 µM (n=5)	54 µM (n=6)	45.9 µM
1,200 mg	0.09-0.16 µM	56 µM (n=2)	147 µM (n=2)	61.3 µM

Table 3. Non-Tumor: Plasma Pentamidine Concentration Ratio

		Randomized Treatment Cohorts				
Variables	**Statistics**	**300 (N=6)**	**600 (N=6)**	**900 (N=6)**	**1,200 (N=4)**	**PBO (N=7)**
Non-Tumor : Plasma Pentamidine Concentration Ratio	N	0	0	1	1	0
	Mean	.	.	14273	12368	.
	Std	.	.	.	.	.
Imputed* Non-Tumor : Plasma Pentamidine Concentration Ratio	N	5	5	6	2	6
	Mean	4003	4344	7826	16877	0
	Std	3552	1932	5384	6377	0

The results of this study showed that VLX103 is truly hepatoselective with adequate hepatic concentrations that increase linearly with dose. Such a feature should be associated with targeted therapeutic effects and a better safety profile vs IV in patients with liver disease such as NASH and alcoholic hepatitis.

5. SUMMARY

VLX103 is the first orally administered pentamidine, designed to target preferentially the liver and be safer than the original IV formulation. In early stage HCC, cirrhotic patients undergoing thermal ablation of their hepatic lesion, VLX103 was shown to be highly hepatoselective, with a linear dose-hepatic concentration relationship in both tumoral and non-tumoral tissue. Hepatic tissue levels ranging from 13 to 145µM were therapeutically relevant, while plasma levels were low or even undetectable at doses ranging from 300 mg to 1,200mg QD x 3 days.

6. REFERENCES

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6. DISCLOSURES

- Patrick Colin & François Ravenelle are employees of Verlyx Pharma Inc.
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