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PHASE I STUDIES

A phase I study of the safety and tolerability of VLX600, an Iron Chelator, in patients with refractory advanced solid tumors

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Summary

Introduction VLX600 is a novel iron chelator designed to interfere with intracellular iron metabolism, leading to inhibition of mitochondrial respiration and bioenergetic catastrophe and resultant tumor cell death. *Methods* We conducted a multicenter, phase 1, dose escalation study to determine the safety and adverse event profile and the maximum tolerated dose and recommended phase 2 dose of VLX600. Other endpoints included pharmacokinetics, and preliminary evidence of anti-cancer efficacy as assessed according to RECIST 1.1 criteria. VLX600 was administered intravenously on days 1, 8, and 15 of each 28-day treatment cycle. *Results* Nineteen patients were enrolled, and seventeen received at least one dose of VLX600. Dose increments were reduced to 50% after dose level 3 (40 mg) due to the occurrence of a grade 3 pulmonary embolism. The study was then closed early due to slow recruitment. No maximum tolerated dose (MTD) nor RP2D had been identified at the time of study closure. Overall, the drug was well tolerated and no DLTs were observed. Fourteen patients experienced drug-related adverse events of any grade. The most frequently reported drug-related AEs were fatigue, nausea, constipation, vomiting, increased alkaline phosphatase, anemia, and decreased appetite. No formal efficacy or survival analyses were performed. No objective responses were observed, though six patients (32%) had stable disease as best response. *Conclusion* VLX600 was reasonably well tolerated and, together with preclinical data, there is support for further efforts to explore its activity as single agent and in combination with drugs or radiation.

Keywords Clinical trial · Phase 1 · Iron chelating agents · VLX600

Introduction

A fundamental problem in cancer drug discovery is the identification of compounds that eliminate dormant malignant cells responsible for tumor relapse. Abnormal vascularization of solid tumors leads to the generation of tissue microenvironments that are chronically starved of oxygen and nutrients [1, 2]. Cells residing in such environments are slowly growing or quiescent and display altered phenotypic characteristics when compared with cells located in more vascularized region [3–5]. These less proliferative cells are often resistant to standard chemotherapeutics that target DNA replication and cell division as their mechanism for antitumor effect [6]. This altered phenotype enables their survival in the face of chemotherapeutics currently utilized, and to reseed nascent tumors in resistance to such chemotherapies and following secession of chemotherapy [7]. Indeed, resistance to a number of agents has been correlated with poor vasculature, tumor relapse and poor patient survival [8]. Thus, there is a critical need to broaden the scope of cancer drug development to focus more on the development of agents that can exploit the altered phenotype of metabolically stressed cells to eliminate tumors.

VLX600 is an iron chelator which differs from that of other well characterized metal chelators such as Dp44mT and Triapine in that VLX600 does not generate reactive oxygen species [9–12]. It interferes with intracellular iron metabolism, leading to inhibition of mitochondrial respiration resulting in bioenergetic catastrophe and tumor cell death. Studies

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demonstrated that, given such a mechanism of action, VLX600 is particularly detrimental under conditions of glucose starvation, with a specific effect on tumor cells populating nutrition-poor microenvironments [13]. VLX600 demonstrated preclinical anticancer activity both in vitro and in vivo. Importantly, the activity of VLX600 was also demonstrated in mice with human colon adenocarcinoma cell line xenografts [13]. Significant antitumor activity was demonstrated in the dose range 0.5–16 mg/kg. Minimal toxicity was observed at doses up to 4.5 mg/kg. At the higher doses, local intolerance problems at the injection site were observed, and mice treated with the highest doses suffered from tremor or decreased motor activity and nervous behavior a few minutes post bolus injection.

The sum of our findings suggested a strategy for targeting the quiescent populations of tumor cells in metabolically compromised microenvironments for improved cancer treatment. Agents with this type of mechanism are expected to be useful in therapeutic combinations with drugs as well as radiotherapy to achieve optimal responses in the treatment of solid tumors. Hence, the rationale for this phase 1 study evaluating VLX600 in humans. This phase 1 study represents the first in human experience with VLX600. This phase 1 trial aimed to investigate the safety and establish the MTD of VLX600 as single drug treatment in patients with advanced solid tumors progressing on or being intolerant to standard drug treatment.

Materials and methods

Study design and treatment

This was a multicenter, phase 1, open label, "3 + 3" design dose escalation study intended to determine the safety, adverse event profile, pharmacokinetics, and maximum tolerated dose (MTD) of VLX600 in patients with refractory advanced solid tumors, to determine a recommended phase 2 dose (RP2D). The study also sought to preliminarily evaluate for anti-cancer activity. VLX600 was reconstituted in 10 mL of sterile water at 30 °C then further diluted with 5% mannitol to final volume of 160 mL with concentration of 1 mg/mL. The volume for a dose was drawn from this solution. Time from start of solution preparation to start of infusion was ≤4 h and dose was administered as an iv infusion over 4 h on days 1, 8, and 15 of each 28-day treatment cycle. The planned dose levels were 10, 20, 40, 60, 90, and 135 mg, with evaluation of intermediate dose levels if necessary. Patients were to receive up to 6 cycles of VLX600 treatment, or until the occurrence of unacceptable toxicity, disease progression, withdrawal of consent or intercurrent illness if prior to 6 treatment cycles. However, patients could continue treatment beyond 6 cycles, if the investigator determined that additional treatment would provide further benefit for the patient.

Patient eligibility

Key inclusion criteria included: (i) ≥ 18 years of age, (ii)) histologic evidence of advanced solid tumors (excluding CNS primary tumors) that were non-resectable, refractory to standard therapies, or patient could not receive or refused standard therapy; (iii) adequate renal (serum creatinine ≤1.5× ULN), hepatic (total bilirubin <1.5× ULN, and AST or ALT ≤3.0× ULN) and hematologic (absolute neutrophil count ≥1.5 × 10 [3]/μL, platelet count ≥100,000, and hemoglobin ≥8.0 g/dL) functions; (v) ECOG performance status of 0 or 1; and (vi) a life expectancy ≥3 months; (iv) measurable disease according to RECIST 1.1 or patients with solid tumors that were not measurable according to RECIST 1.1, but which expressed tumor markers (e.g., prostate cancer with prostate-specific antigen (PSA) expression or ovarian cancer with CA-125 expression). Key exclusion criteria included: (i) uncontrolled intercurrent illness or psychiatric illness/social situations that would limit compliance with study requirements; (ii) prior antineoplastic systemic therapies or radiation therapy ≤4 weeks prior to registration; (iii) CNS metastases not previously treated or not stable for at least 2 months based on imaging and clinical assessment; (iv) failure to fully recover from acute, reversible effects of prior chemotherapy regardless of interval since last treatment; (v) major surgery <28 days prior to study entry; (vi) active other malignancy, except non-melanoma skin cancer or carcinoma-in-situ (e.g., of cervix, breast, prostate); (vii) QTc interval > 450 msec (males) or > 470 msec (females); (viii) clinically relevant retinal abnormalities as per the medical history or ophthalmological findings in the pretreatment evaluation (e.g., retinitis pigmentosa or macular degeneration); (ix) history of thromboembolism within the past 5 years, a history of catheter-related thrombophlebitis or other clinically significant thrombophlebitis.

Dose-limiting toxicity definition

In the study, the following VLX600 doses were to be evaluated: 10, 20, 40, 60, 90, and 135 mg. Patients had to receive 100% of the planned doses during cycle 1 in order to be considered evaluable for tolerability, unless dose reduction, interruption, or discontinuation was the result of a dose limiting toxicity (DLT). The starting dose of VLX600 was 10 mg with planned dose escalations, until a grade 2 study drug-related toxicity was observed. Thereafter, dose escalation was to proceed at no greater than 50% increments until DLT was observed in ≥2 out of 6 patients, and a lower dose level was defined as the MTD. Six additional patients were to be enrolled at the MTD to further evaluate tolerability and antitumor activity.

Safety

Safety assessments were undertaken throughout the study to evaluate AEs, vital signs, body weight, 12-lead electrocardiogram data, concomitant medication use, clinical laboratory values (hematology, blood biochemistry, and urinalysis), and ophthalmological findings. Adverse event (AE) severity was classified using the Common Terminology Criteria for Adverse Events, version 4.0. Ophthalmological examinations were carried out at screening, on day 15 of an odd-numbered cycle, and if clinically indicated.

Pharmacokinetics

Blood samples were collected for PK analysis on day 1 prior to infusion, and then 15, 30, and 60 min after start of infusion (SOI) and 2, 4, 4.25, 4.5, 5, 8 and 24 h after SOI. Samples were also collected on day 8 prior to infusion and then again on day 15 with the same sampling scheme as on day 1. A sample was also taken on day 22 after the last infusion of the first cycle.

Plasma concentrations of VLX600 were determined in human plasma by a validated LC-MS/MS method with a lower limit of quantification of 2.00 ng/mL. Pharmacokinetic analysis of the data was performed using the program PK-Solutions 2.0™ (Summit Research Services and Software, Montrose CO). Non-compartmental pharmacokinetic analysis was applied. The following PK parameters were determined: maximal concentration of the time course (C_{max}) and the corresponding time (T_{max}), area under concentration vs. time curve up to the last detectable concentration, AUC_{last}, AUC_{∞}, mean residence time (MRTarea), apparent volume of distribution, ($Vd_{(area)}$), volume of distribution normalized to body weight (Vd), plasma clearance ($Cl_{(area)}$), and the half-life of the terminal phase ($t½_{,z}$).

Antitumor activity

Antitumor activity was assessed according to RECIST 1.1 criteria [14]. Assessments were carried out within 28 days prior to the start of treatment and every 8 weeks thereafter, or sooner if there was clinical suspicion of disease progression.

Results

Patient characteristics

Nineteen patients (10 female, 9 male), median age 63 years (range: 40–87 years) were enrolled (Table 1**).** All 19 patients were off-study as of data summation and analysis. Two patients were enrolled but did not receive any VLX600, one at dose level 2 (20 mg) and another at dose level 5 (90 mg) due to their withdrawing consent ($n = 1$) or not meeting eligibility criteria (n = 1). Seventeen patients received at least one dose of VLX600 (safety evaluable). Among these patients, median number of doses received was 5.6 and the median number of cycles received was 1.8. All 17 patients who received VLX600 have discontinued VLX600 treatment and are off-study, the primary reason being disease progression.

Table 1 Patient demographics

Gender	
Males	9
Females	10
Age	
Median	63
Range	40–87
Ethnicity	
White	17
Black/ African american	1
Hispanic/Latino	1
Median Duration on Treatment ($n = 17$)	43
Median Number of Doses	5.6
Median Number of Cycles	1.8
Primary Tumor Type	
Colon Adenocarcinoma	4
Pancreas Adenocarcinoma	3
Uterine Adenocarcinoma	2
Hepatocellular Carcinoma	2
Breast Adenocarcinoma	1
Cervix Adenocarcinoma	1
Extragonadal Germ Cell	1
Lung Small Cell Carcinoma	1
Cholangiocarcinoma	1
Appendix Adenocarcinoma	1
Rectal Adenocarcinoma	1
Prostate Adenocarcinoma	1

Dose increments were reduced to 50% after dose level 3 (40 mg) due to the occurrence of a serious adverse event (SAE) of grade 3 pulmonary embolism. Only one of three planned patients were enrolled in the 135 mg dose cohort **(**Table 2**).** The study was then closed early due to slow recruitment. No maximum tolerated dose (MTD) nor RP2D had been identified at the time of study closure.

Safety

Overall, no DLTs were observed. Seventeen patients received at least one dose of VLX600 and the drug was well tolerated at all doses. Fourteen patients experienced drug-related adverse events of any grade (Table 3). The most frequently reported drug-related AEs were fatigue, nausea, constipation, vomiting, increased alkaline phosphatase, anemia, and decreased appetite. With the exception of grade 3 diarrhea,

Table 2 Summary of enrollment per dose level and reasons for study discontinuation

VLX600 enrollment summary							
	VLX600 dose (mg)						
	DL1: 10	DL2: 20	DL3: 40	DL4: 60	DL5: 90	DL6: 135	Overall
Number of patients enrolled	4	4	3	3	4	1	19
Met all eligibility	4	4	3	2	4	1	18
Did not meet all eligibility/waiver granted	0	0	0	1	0	0	1
Received VLX600 treatment	4	3	3	3	3	1	17
Off-study	4	4	3	3	4	1	19
Reason for off-study:							
Progressive disease	2	3	3	3	2	1	14
Withdrawal of consent	0	0	0	0	2	0	2
Investigator decision	1	0	0	0	0	0	1
Other	1	1	0	0	0	0	2

DL = dose level

intestinal obstruction and perforation, anemia, increased AST, increased bilirubin, leukocytosis, increased amylase, hyponatremia, hyperglycemia, hydronephrosis and pulmonary embolism, all other drug-related AEs were mild to moderate (grade 1 or 2) severity. No patients died while on-study or within 30 days of the last study drug dose. One unrelated AE of grade 3 intestinal obstruction in a patient at the 10 mg dose level led to the discontinuation of VLX600. Six serious adverse events (SAEs) were reported in 3 patients; only 1 SAE, grade 3 pulmonary embolism in a patient at the 40 mg dose level was deemed to be drug-related. However, it was suspected that incorrect infusion contributed to the causality as the drug was infused too rapidly, resulting in a rapid increase and peak in drug concentration. The other SAEs were thought to be unrelated or unlikely to be drug-related.

Ophthalmic findings were of particular concern in this study given such observations in preclinical animal testing prior to this clinical trial, though at higher equivalent doses than those being tested here. Dogs showed hypertrophy of retinal pigment epithelium associated with migration of pigmented cells into the subretinal space. Locally extensive retinal atrophy was also documented in some dogs. One patient at the 40 mg dose level was noted on end of treatment ophthalmologic exam to have a small retinal whitened area in the perimacular region. The patient was asymptomatic, and the finding was of unknown significance, but considered possibly explained by drug-related microthrombosis or retinal vein occlusions. Another patient, in the 60 mg dose cohort, was noted to have an asymptomatic "splinter hemorrhage" at the optic nerve of the left eye as a new ophthalmologic finding following the second cycle of VLX600. The ophthalmologist assessed the event as grade 2 splinter hemorrhage possibly related to VLX600 treatment. Splinter hemorrhages can be associated with glaucoma, but glaucoma was absent in this patient. The ophthalmologic examination documentation for both patients who experienced changes was reviewed by an expert ophthalmologist who suggested that these findings may possibly represent drug-related microvasculopathy. Thereafter, investigators were requested to obtain slit-lamp and fundal exams on patients prior to the day 15 dose for each of the next two dose levels of VLX600. No patient at the 90 or 135 mg doses experienced ophthalmologic findings.

Efficacy and survival

Being that the full enrollment was not completed, no formal efficacy or survival analyses were performed. No objective responses were observed, though six patients (32%) had stable disease. Progression of disease was seen in 11 patients (58%) (Table 4). Overall survival, defined as the time from the first day of study drug administration to death due to any cause, ranged from 66 to 499 days.

Pharmacokinetics

Overall, the disposition over the entire dose range studied was dose linear both as regards to Cmax and AUC (Fig. 1a,b). Plasma clearance appeared independent of body weight (Fig. 1c,d). There was no apparent time-dependent difference in the plasma disposition of VLX600. The relationship between plasma clearance obtained after the 1st infusion versus that obtained after the 3rd infusion is seen in Fig. 2. The plasma disposition of VLX600 over the dose range of 10–135 mg was characterized by a multi-exponential elimination (Fig 3a-c).

Table 3 Drug-related adverse events seen

	Grade < 3	Grade >/=3	Total		Grade < 3	Grade	Total		Grade < 3	Grade >/=3	Total
Patients with Adverse Events	10 (58.8%)	7 (41.2%)	17 (100%)	Metabolism and Nutrition	7 (41.2%)	2 (11.8%)	9 (52.9%)	Genitourinary	2 (11.8%)	1 (5.9%)	3 (17.6%)
General	14 (64.7%)	0	14 (64.7%)	Decreased Appetite	4 (23.5%)	0	4 (23.5%)	Hydronephrosis	0	1 (5.9%)	1 (5.9%)
Fatigue	6 (35.3%)	0	6(35.3%)	Hyperglycemia	2 (11.8%)	1 (5.9%)	3 (17.6%)	Urgency	1 (5.9%)	0	1 (5.9%)
Chills	1 (5.9%)	0	1 (5.9%)	Hypocalcemia	2(11.8%)	0	2 (11.8%)	Pollakiuria	1 (5.9%)	0	1 (5.9%)
Malaise	1 (5.9%)	0	1 (5.9%)	Hypernatremia	1 (5.9%)	0	1 (5.9%)	Vascular	3 (17.6%)	0	3 (17.6%)
Edema	1 (5.9%)	0	1 (5.9%)	Hypoalbuminemi	1 (5.9%)	0	1 (5.9%)	Deep Vein Thrombosis (DVT)	1 (5.9%)	0	1 (5.9%)
Chest pain	2 (11.8%)	0	2 (11.8%)	Hyponatremia	0	1 (5.9%)	1 (5.9%)	Hypertension	1 (5.9%)	0	1 (5.9%)
Chills	2 (11.8%)	0	2 (11.8%)	Nervous System	8(47.1%)	0	8 (47.1%)	Lymphedema	1 (5.9%)	0	1 (5.9%)
Pyrexia	2 (11.8%)	0	2 (11.8%)	Dizziness	2 (11.8%)	0	2 (11.8%)	Infectious	2 (11.8%)	0	2 (11.8%)
Local swelling	1 (5.9%)	0	1 (5.9%)	Dysgeusia	2 (11.8%)	0	2 (11.8%)	Onychomycosis	1 (5.9%)	0	1 (5.9%)
Edema	1 (5.9%)	0	1 (5.9%)	Hypoasthesia	1 (5.9%)	0	1 (5.9%)	Upper Respiratory Tract Infection	1 (5.9%)	0	1 (5.9%)
Peripheral edema	1 (5.9%)	0	1 (5.9%)	Migraine	1 (5.9%)	0	1 (5.9%)	Urinary Tract Infection	1 (5.9%)	0	1 (5.9%)
Weight Decreased	1 (5.9%)	0	1 (5.9%)	Peripheral Neuropathy	1 (5.9%)	0	1 (5.9%)	Otologic	1 (5.9%)	0	1 (5.9%)
Night Sweats	1 (5.9%)	0	1 (5.9%)	Restless Legs	1 (5.9%)	0	1 (5.9%)	Ear Pain	1 (5.9%)	0	1 (5.9%)
Gastrointestinal	11 (64.7%)	2 (11.8%)	13(76.5%)	Sinus Headache	1 (5.9%)	0	1 (5.9%)	Reproductive	2 (11.8%)	0	2 (11.8%)
Nausea	9 (52.9%)	0	9 (52.9%)	Visual Field Defect	1 (5.9%)	0	1 (5.9%)	Menorrhagia	1 (5.9%)	0	1 (5.9%)
Constipation	5 (29.4%)	0	5 (29.4%)	MusculosIcektal and Connective Tissue	7 (41.2%)	0	7 (41.2%)	Vaginal hemorrhage	1 (5.9%)	0	1 (5.9%)
Vomiting	4 (23.5%)	0	4 (23.5%)	Limb Pain	3 (17.6%)	0	3 (17.6%)				
Ascites	2 (11.8%)	0	2 (11.8%)	Anthralgia	2(11.8%)	0	2(11.8%)				
Dry Mouth	2 (11.8%)	0	2 (11.8%)	Back Pain	2 (11.8%)	0	2 (11.8%)				
Abdominal Pain	1 (5.9%)	0	1 (5.9%)	Flank Pain	1 (5.9%)	0	1 (5.9%)				
Diarrhea		1 (5.9%)	1 (5.9%)	Muscular Weakness	1 (5.9%)	0	1 (5.9%)				
Dyspepsia	1 (5.9%)	0	1 (5.9%)	Musculoskeletal Pain	1 (5.9%)	0	1 (5.9%)				
Flatulence	1 (5.9%)	0	1 (5.9%)	Respiratory	5 (29.4%)	1 (5.9%)	6 (35.3%)				
Gingival pain	1 (5.9%)	0	1 (5.9%)	Dyspnea	3 (17.6%)	0	3 (17.6%)				
Intestinal Obstruction	0	1 (5.9%)	1 (5.9%)	Cough	2 (11.8%)	0	2 (11.8%)				
Intestinal Perforation	0	1 (5.9%)	1 (5.9%)	Hiccups	1 (5.9%)	0	1 (5.9%)				
Lab Values	6 (35.3%)	4 (23.5%)	10 (58.8%)	Increased Airway Secretion	1 (5.9%)	0	1 (5.9%)				
Increased Alkaline Phosphatase	6 (35.3%)	0	6 (35.3%)	Pulmonary Embolism	0	1 (5.9%)	1 (5.9%)				
Anemia	6 (35.3%)	1 (5.9%)	7 (41.2%)	Wheezing	1 (5.9%)	0	1 (5.9%)				
Aspartate Aminotransferase (AST) Increased	2 (11.8%)	1 (5.9%)	3 (17.6%)	Skin	5 (29.4%)	0	5 (29.4%)				
Alanine Aminotransferase (ALT) Increased	2 (11.8%)	0	2 (11.8%)	Alopecia	2 (11.8%)	0	2 (11.8%)				
Activated Partial Thromboplastin Time (aPTT) Increased	1 (5.9%)	0	1 (5.9%)	Ecchymosis	1 (5.9%)	0	1 (5.9%)				
Amylase Increased	0	1 (5.9%)	1 (5.9%)	Pruritus	1 (5.9%)	0	1 (5.9%)				
Decreased Aspartate Aminotransferase (AST)	1 (5.9%)	0	1 (5.9%)	Seborrheic keratosis	1 (5.9%)	0	1 (5.9%)				
Blood Bilirubin Increased	0	1 (5.9%)	1 (5.9%)	Opthalmologic	2 (11.8%)	0	2 (11.8%)				
Blood Creatinine Increased	1 (5.9%)	0	1 (5.9%)	Splinter Hemorrhage of Retina	1 (5.9%)	0	1 (5.9%)				
Eosinophil Count Increased	1 (5.9%)	0	1 (5.9%)	Retinal exudates	1 (5.9%)	0	1 (5.9%)				
International Normalized Ratio (INR) Increased	1 (5.9%)	0	1 (5.9%)								
Lymphocytosis		1 (5.9%)									
Neutrophil Count Increased	1 (5.9%)	0	1 (5.9%)								
Leukocytosis	1 (5.9%)	0	1 (5.9%)								
Lymphopenia	1 (5.9%)	0	1 (5.9%)								

Table 4 Summary of responses

Disease Response	# of patients (%)
Partial Response (PR)	0 (0%)
Stable Disease (SD)	6 (32%)
Progressive Disease (PD)	11 (58%)

VLX600 was extensively distributed to tissues with a Vd (area) in the range of 1.1–5.1 L/kg. The mean and median of the mean residence time was 10.4 and 9.5 h, respectively, whereas the mean and median elimination half-life was 9.3 and 8.7 h, respectively; all parameters calculated from data from the first infusion. The rest areas were in all instances, except in three profiles, less than 20% of the total area, suggesting adequate sampling duration. However, plasma samples collected prior to the second and third infusion, i.e. 7 days post the 1st and 2nd infusion, showed, in many patients, concentrations above the limit of quantification. Thus, it is reasonable to propose that the terminal elimination half-life of VLX600 is significantly longer than what is reported here. This would not be unexpected owing to the lipophilic character of the compound.

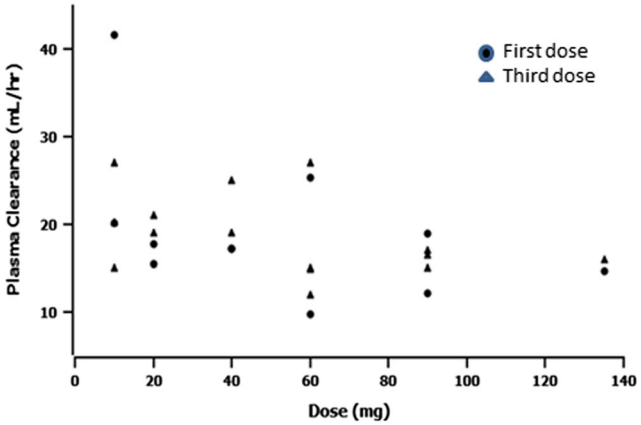

Fig. 2 Relationship between dose plasma clearance of VLX600 in treatment Cycle 1

Discussion

Herein, we report outcomes from the first human experience with VLX600 as a potential anti-cancer therapeutic. The key objectives of this study were to establish the safety, tolerability, DLTs, and preliminary activity of single-agent VLX600 as a treatment for patients with

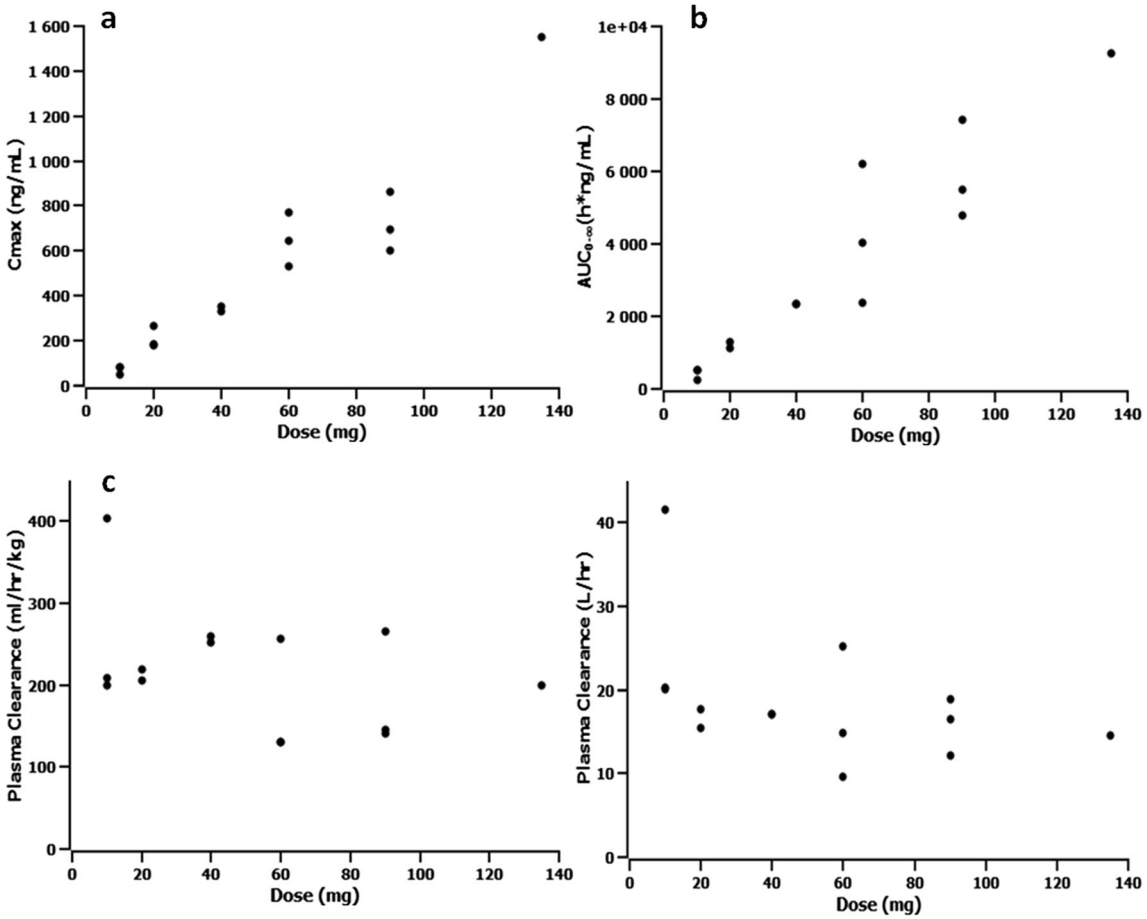

Fig. 1 Relationship between dose and (**a**) Cmax, (**b**)$AUC_{0-\infty}$ (**c**) plasma clearance (mL/hr/kg) and (**d**) plasma clearance (mL/hr) for VLX600 administered as a 4 hr infusion across dose cohorts

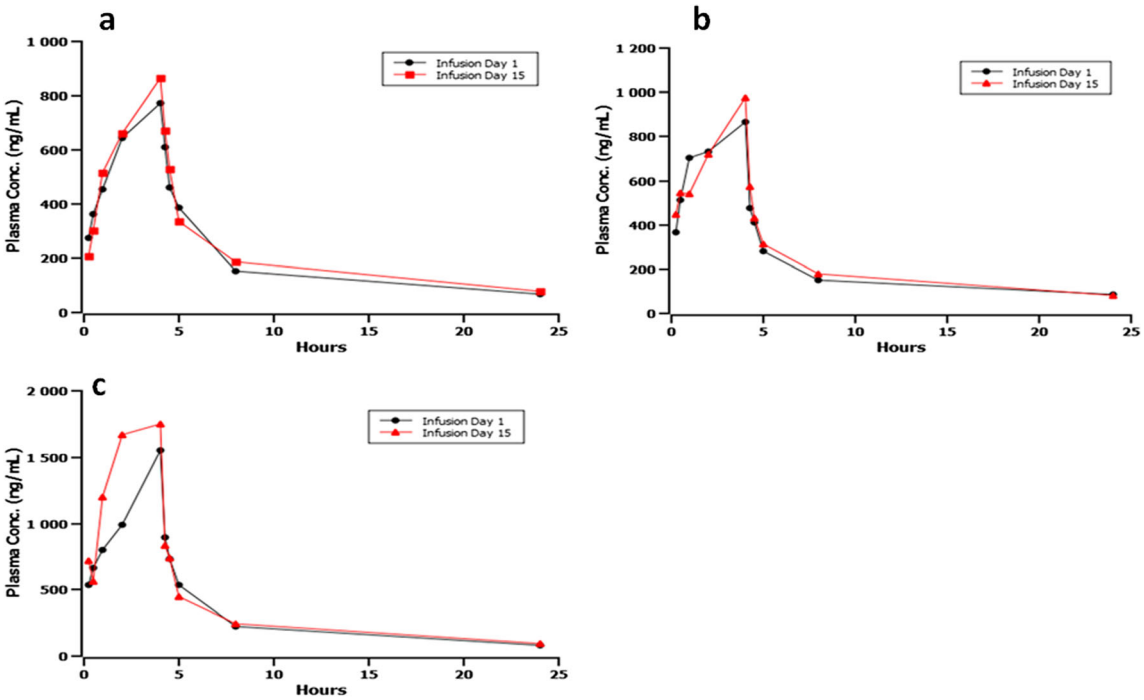

Fig. 3 Plasma concentration time curves of VLX600 in representative patients treated with (**a**) 60mg, (**b**) 90mg, 135mg as a 4 hr infusion

advanced cancers. Clinically meaningful, drug-related nonhematologic and hematologic toxicities of grade > 2 severity were uncommon, no DLTs were encountered, and VLX600 was generally well tolerated.

Iron plays a major role in many crucial physiologic processes, in particular DNA synthesis, cell growth and proliferation, making it an attractive target for anti-cancer chemotherapeutics [15]. Iron primarily works as a co-factor, or as a constituent of co-factors, within the active sites of numerous proteins and enzymes playing critical roles in cellular energy metabolism, DNA synthesis, cell growth and proliferation [15]. Research into iron chelation as a therapeutic strategy against cancer has demonstrated promising novel anti-cancer activity [16]. Depletion of intracellular iron by chelators affects various molecules regulating cell cycle, angiogenesis and metastasis suppression processes. Cellular iron depletion by chelator treatment of breast cancer, leukemia, Kaposi's sarcoma and neuroepithelioma cells, affects the expression and activation of ribonucleotide reductase, cyclins, CDKs and the CDKI, protein 21 (p21), ultimately resulting inhibition of proliferation [17, 18]. Additionally, iron chelators may be preferentially effective in cancer cells versus non-cancerous cells, in part, due to their greater requirement for iron-dependent DNA synthesis [10]. Indeed, one of these processes necessitating iron's involvement is cellular respiration and energy transduction via oxidative phosphorylation and oxygen transport [19]. However, this role of iron means that high and/or improperly sequestered iron can result in the production of reactive oxygen species (ROS) that cause cellular dysfunction. This may in fact be the primary means by which iron chelators result in anti-cancer activity. However, clinically, this could manifest therapeutic toxicity to patients.

Other iron chelators have been explored in the clinic as anti-cancer therapeutics. Dp44mT significantly inhibits growth and metastatic spread of tumors in vitro and in vivo [20, 21]. This was intriguingly demonstrated to be via a p53-independent mechanism, an advantageous feature given that up to 50% of tumors have p53 inactivation [21]. Additionally, systemic iron deficiency was not observed with Dp44mT treatment [21]. In fact, Dp44mT treatment did not result in overall iron depletion in tumors, suggesting instead that the production of reactive oxygen species was the primary anti-tumor mechanism of this class of chelators [21]. While Dp44mT demonstrated significant in vitro and in vivo activity, high non-optimal intravenous doses led to cardiac fibrosis in nude mice [21]. This observation led to a second generation of Dp44mT analogues, one of which is a lead compound for clinical development which is to be determined [11].

Triapine® is another chelator which has demonstrated potent anticancer activity and has been evaluated in a number of clinical trials in patients with a range of cancers. It is a thiosemicarbazone which binds intracellular iron in both the reduced and oxidized states and are potent at inducing cellular iron depletion [22]. However, these agents are also chelators of copper, thus resulting in the formation of redox-active iron and copper complexes which accumulate and produce reactive oxygen species in organelles such as the lysosome [11, 23]. Again, this alone is a significant mechanism of cytotoxicity and cell death, and lends to anti-proliferative activity in cancer cells. Studies in melanoma and breast cancer cells have shown

that the generation of reactive oxygen species may be what is primarily responsible for the ability of Triapine® to inactivate ribonucleotide reductase, its proposed primary mechanism of action [12]. In a trial in patients with metastatic renal carcinoma, enrollment was terminated before completion because of significant adverse effects, notably fatigue, nausea, neutropenia, methemoglobinemia, hypoxia and hypotension [24]. Recent trials evaluating the drug in gynecological malignancies have been more promising, particularly when Triapine® is combined with radiation or other cytotoxics [25].

VLX600 is a novel iron chelator designed to interferes with intracellular iron metabolism, leading to inhibition of mitochondrial respiration and bioenergetics catastrophe and resultant tumor cell death. It differs from other previously characterized metal chelators such as Dp44mT and Triapine in that VLX600 does not generate reactive oxygen species [9–12]. In addition, cytotoxic anticancer activity was observed in vitro and in vivo. In animal models, drug was tolerated well overall.

The enrollment in this study was ceased prior to establishment of an MTD and, thus, few patients were exposed to VLX600 at doses that might be expected to induce tumor remissions. VLX600 was well tolerated and, together with its preclinical properties, there is support for further efforts to explore its activity as single agent and in combination with drugs or radiation for cancer treatment.

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Formal Analysis: KM, MB, AM Data Curation: KM, LV.

Writing – Original Draft: KM, MB, AM Writing – Review & Editing: KM, MB, AM.

Supervision: KM.

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Compliance with ethical standards

Conflict of interest **Kabir Mody**:

Research Support) Senwha Biociences Inc., Tracon Pharmaceuticals, Genentech; Astrazeneca/Medimmune; Arqule, Inc.; Agios; Taiho Oncology; Boston Biomedical; Ipsen.

Consulting/Advisory Board) Eisai Co, Ltd., Bayer Pharmaceuticals.

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Peter Nygren: shareholder in Vivolux AB.

Joachim Gulbo: shareholder in and consultant to Vivolux AB.

All other authors have no relevant conflicts of interest to disclose.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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