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REVIEW ARTICLE

Therapeutic Potential and Utility of Elacridar with Respect to P-glycoprotein Inhibition: An Insight from the Published In Vitro, Preclinical and Clinical Studies

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Abstract The occurrence of efflux mechanisms via Permeability-glycoprotein (P-gp) recognized as an important physiological process impedes drug entry or transport across membranes into tissues. In some instances, either low oral bioavailability or lack of brain penetration has been attributed to P-gp mediated efflux activity. Therefore, the objective of development of P-gp inhibitors was to facilitate the attainment of higher drug exposures in tissues. Many third-generation P-gp inhibitors such as elacridar, tariquidar, zosuquidar, etc. have entered clinical development to fulfil the promise. The body of evidence from in vitro and in vivo preclinical and clinical data reviewed in this paper provides the basis for an effective blockade of P-gp efflux mechanism by elacridar. However, clinical translation of the promise has been elusive not just for elacridar but also for other P-gp inhibitors in this class. The review provides introspection and perspectives on the lack of clinical translation of this class of drugs and a broad framework of strategies and considerations in the potential application of elacridar and other P-gp inhibitors in oncology therapeutics.

Key points

P-glycoprotein inhibitors (P-gp) are gaining momentum in the present-day drug therapy because these provide an opportunity for tissue-specific accumulation of drug by overcoming the natural or acquired efflux mechanism(s) of the local environment.

Many third-generation P-gp inhibitors such as tariquidar, elacridar, zosuquidar, laniquidar, ONT-093, etc. have advanced to clinical development as a combination therapy in different therapeutic areas most notably in the cancer area.

While strong evidence reported from in vitro and in vivo preclinical and clinical data reviewed in this paper provide the mechanistic basis for an effective blockade of P-gp efflux by elacridar, the clinical translation of the promise has been elusive not just for elacridar but also for other third-generation P-gp inhibitors.

A number of critical questions and key development strategies need to be considered to understand and overcome the observed dilemma of lack of clinical translatability of this class of P-gp inhibitors.

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1 Introduction

During the past 2 decades, researchers have identified many membrane transporters specifically related to drug pharmacokinetics and disposition [1]. These transporters

play a crucial role in the absorption, distribution and elimination of drugs and their metabolite(s). Initially, the role of the transporters was established for drug excretion and subsequently identified in the drug absorption process [2]. The solute carriers (SLC) and ATP-binding cassette (ABC) transporters are considered as the primary classes of drug transport proteins that are widely distributed in different tissues in the body and exhibit broad substrate specificity [3]. These transporters modulate the access of the drugs into the cells and thus control the subsequent pharmacological and toxicological effects. They are classified as influx and efflux transporters based on their direction of substrate translocation. ABC transporters are considered as primary efflux transporters that use energy generated from adenosine triphosphate (ATP) hydrolysis to transport the substrate from intracellular to extracellular locale, mostly against a concentration gradient [2]. Amongst the ABC transporters, P-glycoprotein (P-gp) plays a significant role in modulating the drug uptake and access to different tissues. P-gp, a glycosylated membrane protein consists of 1280 amino acids with 12 hydrophobic, helical transmembrane segments, two intracellular ATP binding sites and a molecular weight of 170 Da. P-gp was first identified in cancer cells and later found in normal tissues such as the apical membrane of intestinal epithelial cells, the biliary cannalicular membrane of liver, the luminal membrane of proximal tubular epithelial cells in kidney and the luminal membrane of the endothelial cells forming the blood–brain barrier (BBB), blood-cerebro spinal fluid barrier (BCSFB), and blood-testis barrier [4–8]. P-gp located across the BBB limits the access of the drugs/xenobiotics into the central nervous system (CNS). Intestinal P-gp effluxes some of the drugs administered orally, thus reducing the bioavailability [9].

Several approaches were followed to overcome the issue of P-gp-mediated reduced oral bioavailability of important drugs that included developing suitable dosage form using nanotechnology, use of alternative route of drug administration and co-administration of P-gp inhibitors [10].

P-gp inhibitors are classified into three generations based on their specificity and affinity [2]. The first-generation inhibitors include several commonly used drugs such as verapamil, cyclosporin A, reserpine, quinidine, yohimbine and tamoxifen [2]. However, the major drawback of this class of P-gp inhibitors is the dose-related toxicity to achieve desirable P-gp inhibition. Second generation P-gp inhibitors, namely non-immunosuppressive analogues of cyclosporin A, PSC 833; D-isomer of verapamil, dexverapamil; and others such as biricodar (VX-710), GF120918 and MS-209 were devoid of the pharmacological activity and exhibited higher affinity as compared to the first-generation inhibitors. Another major limitation of second-generation P-gp inhibitors is their non-specific interaction with cytochrome P450 (CYP) enzymes [11]. However, third-generation inhibitors are found to be more specific and are regarded to display a better toxicity profile. Various third-generation inhibitors those are under development include tariquidar, elacridar, zosuquidar, laniquidar and ONT-093. The third-generation inhibitors do not interact with CYP enzymes; however, some interaction has been observed with other transporters outside of both P-gp and breast cancer resistance protein (BCRP) [11, 12]. These P-gp inhibitors show their activity by (a) blocking drug binding site either competitively, non-competitive or allosterically and (b) interfering with the ATP hydrolysis and (c) by altering integrity of cell membrane lipids [13, 14].

Amongst the third-generation P-gp inhibitors, elacridar has been extensively investigated. The chemical name of elacridar (also known as GF120918) is (*N*-[4-[2-(3,4-Dihydro-6,7-dimethoxy-2(1H)-isoquinolinyl)ethyl]phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridinecarboxamide). It is a white coloured powder with a molecular weight of 563.64 and exhibits a solubility of 2 mg/ml in dimethyl sulfoxide (DMSO) [11]. Elacridar is a potent and specific non-competitive inhibitor of P-gp [11]. Elacridar acts by inhibiting the ATP hydrolysis by modulating the ATPase activity [15].

Along with elacridar, two other P-gp inhibitors, namely tariquidar and zosuquidar, are currently under clinical development [11]. Tariquidar binds specifically and non-competitively to the P-gp and has exhibited dose linearity in systemic exposure in healthy male subjects. Clinical pharmacokinetic study in healthy subjects suggest that the maximum plasma concentration (C_{max}) was 2.3 μM, area under the curve ($AUC_{0–48}$) was 12.6 μM h, clearance was 0.19 l/h/kg, volume of distribution was 246 l/m^2 and the terminal elimination half-life was 26 h, at a dose of 2 mg/kg [16].

Zosuquidar is characterised by high potency and low toxicity and has entered the clinical trial in combination with vinorelbine and doxorubicin for various types of advanced malignancies [17, 18]. Zosuquidar has been reported to be the most specific of the third-generation inhibitors of ABCB1 with little measurable effect on ABCG2 or ABCC1 transporters, or CYP in vitro [17]. Pharmacokinetic study in cancer patients suggested that the clearance of zosuquidar is independent of the dose. The half-life ($t_{1/2}$) following intravenous administration of 640 mg/m^2/day was found to be 17 h, suggesting slow elimination [18]. However, the major reported side-effect is neurotoxicity and potential drug–drug interactions with vinorelbine and doxorubicin [19]. P-gp inhibitors are gaining significant importance in drug development because of the promise of avoiding efflux mechanism to ensure higher attainment of drug exposures in the desired regions of the body [20–22]. Table 1 summarizes the

Table 1 Clinical development status of third generation P-gp inhibitors

Compound (sponsor)	Trial number	Approval/development status	References
Tariquidar/XR9576 (QLT Inc.)	NCT00048633	Phase II	[111]
Zosuquidar (Eastern Cooperative Oncology Group)	NCT00046930	Phase III	[112]
Laniquidar	NCT00028873	Phase II	[113]
(European Organisation for Research and Treatment of Cancer—EORTC)			
ONT-093 (Ontogen)	NA	Early clinical	NA
Elacridar (GlaxoSmithKline)	NA	Early clinical	NA

clinical development status of third-generation P-gp inhibitors.

Increasing efforts are also underway to develop novel P-gp inhibitors that exhibit higher specificity and affinity along with minimal toxicity to overcome the issues of multi-drug resistance and compromised drug bioavailability.

2 Scope

As elacridar has shown promise as a P-gp inhibitor, this review compilation was instituted to understand its pharmacokinetic aspects. The focus of this review is towards compilation of nonclinical and clinical pharmacokinetics of elacridar and critically probe the drug development considerations of this class of P-gp inhibitors using elacridar as the probe. The literature review was done using Pubmed® search (NCBI 2016), SCIFINDER® and Google Scholar databases with specific key words such as P-gp inhibitor, elacridar, preclinical, clinical, pharmacokinetics, absorption, distribution, metabolism, excretion, bioavailability, disposition, drug–drug interaction, transporters, enzymes, animal and human to collect the related full-length articles and abstracts.

This review is organized to provide the following: (a) an overarching compilation of the in vitro P-gp inhibitory potential exhibited by elacridar applicable to various therapeutic areas; (b) a tabular summary of the status of P-gp inhibitors in drug development; and (c) summarize the reported preclinical and clinical pharmacokinetic studies of elacridar (Tables 2, 3). The individual tabular pharmacokinetic summary was designed to succinctly capture study designs, objectives and evaluable pharmacokinetic parameters with key remarks. Additionally, a discussion section provides perspectives; strategies and considerations in the applicability of elacridar and/or this class of drugs for potential use in oncology therapies where P-gp inhibition may show benefits.

3 In Vitro P-gp Inhibition Activity

3.1 Anticancer Therapy

O'Neill et al. observed that elacridar increased the cellular uptake of docetaxel in resistant DU-145 R (moderate P-gp expression) and 22RV1 R (high P-gp expression) prostate cancer cell lines, but it was not the case with respect to the resistant PC-3 cell lines that were devoid of P-gp expression. Thus, it may be inferred that multiple mechanisms contribute towards docetaxel resistance including P-gp efflux [23].

Elacridar increased the response of hepatoblastoma cell line (i.e., HepT1) to facilitate the treatment with doxorubicin. The IC_{50} for doxorubicin + elacridar was 1.7 times lower as compared to native doxorubicin [24]. Another study suggested that the cellular uptake of doxorubicin from a formulated doxorubicin + elacridar (polymer-lipid hybrid nanoparticles) as well as non-formulated native doxorubicin + elacridar was more than 1.5 times higher as compared to the cellular uptake of native doxorubicin alone [25].

Marchetti et al. described the impact of elacridar on the cellular uptake of topotecan and gimetecan in different breast cancer cell lines. The IC_{50} of topotecan + elacridar in T8 and MDCKII-BCRP1 cell lines were 32 and 57 times lower as compared to native topotecan. Similarly, gimetecan + elacridar exhibited 3 and 6 times lower IC_{50} in T8 and MDCKII-BCRP1 cell lines as compared to native gimetecan [26].

The studies in hepatocellular carcinoma cell lines showed that elacridar increased the cellular uptake of irinotecan and its metabolite SN-38 in KYN-2 (expressing BCRP, CYP3A4/5 and UGT1A1) and KYN-1 cell lines (expressing BCRP only), thus suggesting that BCRP is one of the chemo-sensitivity determinants of irinotecan in hepatocellular carcinoma cell lines and its inhibition might be critical for cells expressing abundant BCRP [27].

The intestinal absorptive and secretory transport of irinotecan was investigated using Caco-2 cell monolayers

Table 2 Summary of study design, objectives and pharmacokinetic data of elacridar monotherapy and drugs co-administered with elacridar in preclinical pharmacology studies

Drug (references)	Study particulars		Pharmacokinetic data							Remarks
	Species, strain, sex and route of administration	Formulation (dosage)	Tissue/plasma	C_{max} (μg/g or μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg·h/g or μg·h/mL)	CL or CL/F (L/h)	V_d (L)	
Elacridar [50]	Mouse, rat, dog and monkey	Suspensions as 0.5% aqueous HPMC containing 1% Tween 80 at 3 and 30 mg/kg dose	Plasma							Linear dose-concentration relationship could be established across the tested dose levels
			Mouse							
			3 mg/kg	0.086 ± 0.024	2		1.602 ± 0.252			
			30 mg/kg	0.586 ± 0.025	4		19.14 ± 4.86			
			Rat							
			3 mg/kg	0.018 ± 0.004	2		0.399 ± 0.090			
			30 mg/kg	0.133 ± 0.029	4		5.994 ± 1.69			
			Dog							
			3 mg/kg	0.125 ± 0.030	6		5.82 ± 2.40			
			30 mg/kg	0.219 ± 0.050	6		12.12 ± 4.68			
			Monkey							
			3 mg/kg	0.086 ± 0.037	4		2.42 ± 0.67			
			30 mg/kg	0.179 ± 0.100	6		11.22 ± 4.32			
Topotecan [57]	Female wild-type mice. Topotecan administered intravenously and elacridar orally	Topotecan (5 mg/kg) was dissolved in glucose (5%; w/v). Elacridar (100 mg/kg) suspended at 5 mg/mL in a mixture of hydroxypropyl methylcellulose (0.5 g/L)/1% polysorbate 80 (v/v)	Topotecan alone							Elacridar increased the brain-to-plasma ratio for topotecan
			Plasma				0.475 ± 0.049			
			Brain				0.150 ± 0.019			
			Liver				4.807 ± 0.610			
			Topotecan + Elacridar							
			Plasma				1.332 ± 0.093			
			Brain				0.680 ± 0.045			
			Liver				16.111 ± 1.328			
Docetaxel [60]	Wild-type mice. Docetaxel administered intravenously and elacridar orally	Docetaxel (33 mg/kg) formulated in Tween 80:ethanol:saline (20:13:67, v/v/v). Elacridar (25 mg/kg) prepared in 5 g/l of hydroxypropyl methyl Cellulose and 1% Tween 80	Docetaxel alone							Elacridar increased the oral exposure of docetaxel significantly
			Plasma				8.7 ± 0.9			
			Brain				2.5 ± 0.2			
			Liver				40.6 ± 5.4			
			Docetaxel + Elacridar							
			Plasma				12.4 ± 1.4			
			Brain				9.1 ± 1.0			
			Liver				60.6 ± 6.4			
Pazopanib [64]	Male FVB wild-type mice. Pazopanib and elacridar administered intravenously	Pazopanib (5 mg/kg) and elacridar (10 mg/kg) were dissolved in 3:4:3, DMSO:propylene-glycol:0.9% saline (v/v/v)	Pazopanib alone							Elacridar enhanced pazopanib brain penetration by ~fivefold without altering its plasma concentrations
			Plasma			1.39	81,360	0.1428	0.29	
			Brain			1.89	1200			
			Pazopanib + Elacridar							
			Plasma			1.83	61,740	0.1494	0.40	
			Brain			2.75	2520			

Table 2 continued

Drug (references)	Study particulars		Pharmacokinetic data						Remarks
	Species, strain, sex and route of administration	Formulation (dosage)	Tissue/plasma	C_{max} (μg/g or μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg·h/g or μg·h/mL)	CL or CL/F (L/h)	V_d (L)	
Imatinib [76]	Wild-type mice. Imatinib and elacridar administered orally	Imatinib (12.5 mg/kg) was diluted with glucose 5%. Elacridar (30 mg/kg) was suspended at 10 mg/mL in a mixture of hydroxypropylmethylcellulose (10 g/L)/2% Tween 80/H_2O [0.5:1:98.5 (v/v/v)]	Plasma							Elacridar increased the oral bioavailability of imatinib by 3.3-fold
			Imatinib	10.8		2.0	30.0			
			Imatinib + Elacridar	11.8		4.1	99.3			
Sorafinib [82]	FVB wild-type mice. Sorafinib and elacridar administered intravenously	Sorafinib (10 mg/kg) administered in DMSO, propylene glycol and saline (3:5:2 v/v/v). Elacridar (10 mg/kg) dissolved in 10:7:3 v/v/v DMSO, propylene glycol, saline	Sorafinib							Elacridar increased the brain exposure of sorafinib by more than 7 times without altering the plasma concentration
			Plasma	3.9						
			Brain	0.52						
			Sorafinib + Elacridar							
			Plasma	4.04						
			Brain	3.9						
Vandetanib [84]	Male FVB wild-type mice. Pazopanib and elacridar administered intravenously	Vandetanib (5 mg/kg) and elacridar (10 mg/kg) were dissolved in 3:4:3, DMSO:propylene-glycol:0.9% saline (v/v/v)	Vandetanib alone							Elacridar enhanced Vandetanib brain penetration by ~threefold without altering its plasma concentrations
			Plasma			1.60	18,300 ± 732	0.5616	1.30	
			Brain			1.65	3774 ± 48			
			Vandetanib + Elacridar							
			Plasma			1.58	16,578 ± 690	0.6198	1.41	
			Brain			4.28	10,638 ± 1134			
Convallatoxin[a] [91]	Male wistar rats. Convallatoxin and elacridar administered intravenously	Convallatoxin (1.25 mg/kg) with or without elacridar (6 mg/kg) were dissolved in ethanol/PEG400/5%glucosesolution(20:60:20)	Convallatoxin alone							Elacridar increased the brain exposure of convallatoxin, significantly
			Plasma	0.26 ± 0.10						
			Brain	0.5 ± 0.2						
			Convallatoxin + Elacridar							
			Plasma	0.39 ± 0.05						
			Brain	1.0 ± 0.2						
GV196771 [93]	Male wild-type mice. Drugs administered orally	GV196771 (2 mg/kg) and elacridar (50 mg/kg) administered as a suspension in 0.5% hydroxypropy lmethyl cellulose and 1% Tween 80	Plasma							Elacridar increased the systemic exposure of GV196771 by 10-times
			GV196771	0.106	0.5		0.184			
			GV196771 + Elacridar	1.463	0.5		1.886			
Itraconazole [98]	BALB/c mice infected with *Cryptococcus neoformans*. Drugs administered intraperitoneally	Itraconazole (10 mg/kg) was administered by diluting the parenteral injection 5 times, whereas elacridar (10 mg/kg) was dosed in PEG/water (25/75, v/v)	Plasma							Pretreatment with elacridar significantly increased cerebral itraconazole levels
			Itraconazole	1.85	1.0	1.65	6.29	0.046	0.164	
			Itraconazole + Elacridar							
			Brain	2.59	0.5	2.72	6.41	0.042	0.110	
			Itraconazole	0.75	0.5	0.43	2.49			
			Itraconazole + Elacridar	1.31	0.5	0.37	3.89			

Table 2 continued

Drug (references)	Study particulars		Pharmacokinetic data						Remarks
	Species, strain, sex and route of administration	Formulation (dosage)	Tissue/plasma	C_{max} (μg/g or μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg·h/g or μg·h/mL)	CL or CL/F (L/h)	V_d (L)	
Loperamide [99]	Male Sprague–Dawley rats. Drugs administered intravenously	Loperamide (0.5 mg/kg) was dissolved separately in a mixture of saline and PEG 600 (3:1). Elacridar (1.0 mg/kg) was dissolved in tetraglycol	Brain							Elacridar increased the brain levels of loperamide by 3.5-fold
			Loperamide	5.052 ± 0.8	1.0	3.6 ± 0.3	28.90 ± 4.55	0.0044 ± 0.001	0.022 ± 0.003	
			Loperamide + Elacridar	5.855 ± 0.626	1.0	4.9 ± 0.7	34.41 ± 4.31	0.0034 ± 0.001	0.021 ± 0.004	

Data expressed as mean ± SD (wherever reported), except for t_{max} which are expressed as median

AUC area under the curve, *Cl/F* total body clearance, C_{max} maximum plasma concentration, *IV* intravenous, $t_{1/2}$ half-life, V_d volume of distribution

[a] Data expressed as nM

and engineered Madin-Darby canine kidney (MDCK) II cells overexpressing P-gp, cannalicular multi-specific organic anion transporter (cMOAT) and MRP1 [28]. Elacridar (IC_{50}—0.38 ± 0.06 μM) significantly decreased the secretory efflux of irinotecan [28].

Elacridar increased the sensitivity of ixabepilone, a novel microtubule targeting agent in MDCK and MDCK-MDRI cell lines. The IC_{50} of elacridar + ixabepilone was 90 times lower as compared to native ixabepilone [29]. Xia et al. observed that elacridar increased the apical-to-basolateral (A-to-B) transport and decreased the basolateral-to-apical (B-to-A) transport of methotrexate in Caco-2 cells [30].

Mitoxantrone uptake was 4.6 times higher in the presence of elacridar (5 μM/L) when evaluated in the human choriocarcinoma cell line BeWo, an in vitro model of the human trophoblast [31]. Tallkvist et al. observed that elacridar reduced secretion and increased accumulation of mitoxantrone in both undifferentiated and differentiated mammary epithelial HC11 cells [32]. The effect of elacridar on the sensitivity of human IGROV-1 ovarian cancer cell line and its cisplatin resistant variant IGROVCDDP towards paclitaxel, docetaxel and epirubicin was observed. The results showed that IC_{50} of elacridar + paclitaxel, elacridar + docetaxel and elacridar + epirubicin were 1.54, 61 and 2.6 times lower as compared to native paclitaxel, docetaxel and epirubicin in IGROV-1 cell lines. However, for IGROVCDDP cell lines, the IC_{50} of elacridar + paclitaxel, elacridar + docetaxel and elacridar + epirubicin were 404, 102,812 and 129 times lower as compared to native paclitaxel, docetaxel and epirubicin [33]. O'Conner et al. observed the potentiating effect of elacridar when bortezomib and elacridar combination was evaluated in several different P-gp-resistant cancer cell lines, including DLKP-A (lung cancer) NCI-Adr/res (ovarian cancer) and RPMI-Dox40 (MM) cells. The findings of the study showed that bortezomib is a substrate of P-gp, and this resistance was greatly reduced when P-gp efflux was inhibited by elacridar [34]. Elacridar increased the intracellular accumulation of CGP74588, a pharmacologically active metabolite of imatinib by 5 times in rat C6 glioma cells [35]. Sato et al. observed that the sunitinib resistance could be reversed by the co-treatment with elacridar in renal carcinoma cell lines 786-O; however, similar outcome was not observed in ACHN and Caki-1 cell lines [36].

3.2 Antiretroviral Area

Neumanova et al. observed that elacridar increased the apical-to-basolateral (A-to-B) transport and decreased the B-to-A transport of abacavir in MDCK-II cell lines [37]. A significant decrease in the B-to-A transport was observed in

Table 3 Summary of study design, objectives and pharmacokinetic data of drugs co-administered with elacridar in human clinical pharmacology studies

Drug [References]	Study particulars		Pharmacokinetic data						Remarks
	Subjects/design	Objective (s)	Type	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg·h/mL)	CL or CL/F (L/h)	V_d (L)	
Doxorubicin [105]	$N = 46$ patients; 7 cohorts; phase I Course 1: Single-dose doxorubicin (50–75 mg/m^2) on Day 1 Course 2: Doxorubicin (50–75 mg/m^2) + Elacridar (50 mg q.d. to 400 mg b.i.d) on Day 22–24 Course 3: Doxorubicin (50–75 mg/m^2) + Elacridar (50 mg q.d. to 400 mg b.i.d) on Day 31 Blood samples were collected up to 48 h, following doxorubicin administration. Samples for elacridar analysis were collected up to 24 h	Determining the pharmacokinetics of doxorubicin in patients with advanced solid tumors in the presence and absence of elacridar.	Cohort 1 ($n = 5$); DOX 50 mg/m^2 + ELA 50 mg q.d.						Elacridar increased the plasma exposure of doxorubicinol to reverse MDR
			DOX	3.05 ± 1.23		0.13 ± 0.04	1.67 ± 0.353		402 ± 77	
			DOX + ELA	2.97 ± 0.91		0.11 ± 0.04	1.55 ± 0.361		460 ± 99	
			DOXOL	0.033 ± 0.011			0.539 ± 0.331			
			DOXOL + ELA	0.046 ± 0.016			0.580 ± 0.286			
			Cohort 2 ($n = 4$); DOX 50 mg/m^2 + ELA 50 mg b.i.d.							
			DOX	2.13 ± 1.09		0.12 ± 0.05	1.33 ± 0.414		550 ± 269	
			DOX + ELA	1.89 ± 1.10		0.14 ± 0.05	1.31 ± 0.281		779 ± 278	
			DOXOL	0.052 ± 0.033			0.416 ± 0.175			
			DOXOL + ELA	0.046 ± 0.027			0.695 ± 0.319			
			Cohort 3 ($n = 5$); DOX 50 mg/m^2 + ELA 100 mg b.i.d.							
			DOX	3.24 ± 0.61		0.10 ± 0.01	1.66 0.566		595 ± 188	
			DOX + ELA	3.25 ± 1.02		0.08 ± 0.01	1.62 ± 0.352		650 ± 149	
			DOXOL	0.041 ± 0.014			0.506 ± 0.136			
			DOXOL + ELA	0.048 ± 0.016			0.635 ± 0.228			
			Cohort 4 ($n = 5$); DOX 50 mg/m^2 + ELA 200 mg b.i.d.							
			DOX	3.70 ± 1.02		0.09 ± 0.01	1.26 0.296		798 ± 293	
			DOX + ELA	3.64 ± 1.03		0.09 ± 0.01	1.62 ± 0.363		488 ± 319	
			DOXOL	0.038 ± 0.015			0.493 ± 0.199			
			DOXOL + ELA	0.046 ± 0.019			0.738 ± 0.322			
			Cohort 5 ($n = 5$); DOX 50 mg/m^2 + ELA 400 mg bi.d.							

Table 3 continued

Drug [References]	Study particulars		Pharmacokinetic data						Remarks
	Subjects/design	Objective (s)	Type	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg·h/mL)	CL or CL/F (L/h)	V_d (L)	
			DOX	2.49 ± 0.38		0.09 ± 0.02	1.39 ± 0.327		733 ± 260	
			DOX + ELA	3.56 ± 1.71		0.12	1.72 ± 0.324		932 ± 390	
			DOXOL	0.038 ± 0.006			0.651 ± 0.323			
			DOXOL + ELA	0.055 ± 0.015			1.35 ± 0.648			
			Cohort 6 ($n = 4$); DOX 60 mg/m^2 + ELA 400 mg b.i.d.							
			DOX	3.66 ± 0.97		0.09 ± 0.02	1.85 ± 0.245		762 ± 207	
			DOX + ELA	3.93 ± 1.55		0.09 ± 0.02	1.99 ± 0.233		725 ± 151	
			DOXOL	0.026 ± 0.003			0.743 ± 0.058			
			DOXOL + ELA	0.033 ± 0.005			0.969 ± 0.244			
			Cohort 7 ($n = 4$); DOX 75 mg/m^2 + ELA 400 mg b.i.d.							
			DOX	3.11 ± 1.40		0.10 ± 0.01	1.70 ± 0.398		1040 ± 254	
			DOX + ELA	3.90 ± 0.77		0.10 ± 0.01	2.57 ± 0.295		983 ± 187	
			DOXOL	0.039 ± 0.007			0.810 ± 0.201			
			DOXOL + ELA	0.056 ± 0.019			2.11 ± 0.911			
Paclitaxel[a] [106]	$N = 6$ cancer patients Treatment: Paclitaxel orally 120 mg/m^2 + single oral dose of GF120918 (1000 mg) Blood samples were collected to up to 48 h, following paclitaxel administration	Determining the effect of elacridar on oral bioavailability of paclitaxel	Paclitaxel + Elacridar	0.36 ± 0.17	2.8		3.27 ± 1.67			Elacridar increased the oral exposure of paclitaxel significantly

Data expressed as mean ± SD (wherever reported), except for t_{max} which are expressed as median

DOX doxorubicin, *ELA* elacridar, *DOXOL* doxorubicinol, *q.d* once daily, *b.i.d* twice daily, *AUC* area under the curve, *CL/F* total body clearance, C_{max} maximum plasma concentration, *IV* intravenous, $t_{1/2}$ half-life, V_d volume of distribution

[a] Data expressed as μM

MDCK–MDR1 for zidovudine and MDCK–MDR1 and Caco-2 for lamivudine [38].

3.3 Miscellaneous

The findings from in vitro trans-well assays suggested that elacridar completely inhibited the efflux of EPZ-6438, an inhibitor of Enhancer of Zeste Homolog 2 (EZH2) and implicated in multiple gliomas and increased the brain penetration [39]. Elacridar increased the intracellular accumulation of kaempferol by 15, 2.11 and 1.5 times at concentrations of 5, 10 and 15 μM, respectively, in MDCK-II cell lines thus indicating that kaempferol is a substrate for P-gp. However, it was important to note that no dose-linear relationship was observed with respect to efflux ratio [40]. Sugano et al. described that elacridar (0.5 μM/L) reversed the resistance of non-small cell lung cancer cell line EBC-1R towards PHA-665752, a MET inhibitor by inhibiting the cancer-stem cells like property that leads to activation of ABCB1 [41]. Elacridar (5.0, 1.0 and 0.2 μM) decreased the efflux and increased the absorption of Ochratoxin A (OTA), a major secondary metabolite formed by various fungal species of the genus *Penicillum* and *Aspergillus* in Caco-2 cell model [42].

Elacridar decreased the efflux of danofloxacin in a dose-dependent manner (0.04–5 μM/L). A 1.5 times decrease in the efflux was observed at a dose of 5 μM/L as compared to native danofloxacin [43]. Miller et al. described that elacridar decreased the ATPase concentration and efflux of darifenacin in MDCK-MDRI cell lines [44]. Elacridar also reduced the basolateral to apical permeability of digoxin in MDCK-MDR1, Caco-2 and CPT-B2 cell lines [45]. The resistance of *Leishmaniasis* cell line DNM-R150 towards miltefosine was reversed by elacridar (1 μM). A 30% inhibition in the growth and 50% reversal were observed in the presence of elacridar [46]. An investigation in sandwich-cultured human hepatocytes revealed that co-incubation of tolvaptan with elacridar (10 μM) reduced DM-4107 (metabolite of tolvaptan) accumulation by 23.0% relative to control, with no effect on the accumulation of tolvaptan, thus suggesting that elacridar might have facilitated the cannalicular transport and modulated CYP enzymes in the human hepatocytes [47].

4 Ex-vivo P-gp Inhibition Activity

The role of placental P-gp with respect to maternal to fetal transfer was evaluated for L-α-acetylmethadol (a congener of methadone that has been used for treatment of the adult opiate addict) and paclitaxel using human placental lobule. The results suggested that the fetal rate transfer, maternal clearance and clearance index for L-α-acetylmethadol were 1.23, 1.16 and 1.26 times higher, respectively, in the presence of elacridar as compared to the control group [48]. Similarly, elacridar increased the fetal rate transfer, maternal clearance and clearance index by 2.0, 2.0 and 1.75 times, respectively, for paclitaxel as compared to the control group [48]. Elacridar increased the absorptive permeability of digoxin by 3.3 times when evaluated using the rat intestinal brush-border vesicles [49].

5 Pharmacokinetic Properties of Elacridar

5.1 Preclinical Pharmacokinetics

5.1.1 Absorption

Ward and Azzarano described the pharmacokinetic profile of elacridar in mouse, rat, dog and monkey. The studies were conducted at 3 and 30 mg/kg for all the species, whereas additional experiment was conducted in mice and rats at 300 mg/kg. Linear dose–concentration relationship could be established across the tested dose levels [50]. Sane et al. observed that the absolute bioavailability of elacridar in mice was 0.22 for oral administration and 0.01 for intra-peritoneal administration. Low aqueous solubility and high lipophilicity of elacridar are considered responsible for poor oral absorption [51].

5.1.2 Distribution

Plasma protein binding was found to be 98.5, 99.0, 99.8, 99.9 and 99.9 for mouse, rat, dog, monkey and human, respectively [50]. Biodistribution study in mice showed that the brain-to-plasma partition coefficient of elacridar in the wild-type mice was 0.82, as compared with 3.5 in MDR1a/b(−/−) mice indicating that P-gp limits the brain distribution of elacridar [52]. The brain-to-plasma partition coefficient after intravenous, intra-peritoneal and oral dosing was 0.82, 0.43 and 4.31, respectively [51].

5.1.3 Metabolism

Metabolism study using human P450 enzymes such as CYP1A2, 2C9, 2C19, 2D6 AND 3A4 showed that elacridar is not a potent inhibitor of CYP enzymes. The IC_{50} value ranged from 10.5 to 49.9 μM across all the tested enzymes [50].

5.1.4 Elimination

Elacridar was observed to be eliminated with a modest half-life of 3–6 h in all the preclinical species and appeared to be dose independent [50]. The mean hepatic extraction

at 3 mg/kg dose for rat, dog and monkey were found to be 57.0, 24.1 and 76.2, respectively, whereas at 30 mg/kg dose was 40.5, 23.4 and 76.4, respectively [50].

5.2 Clinical Pharmacokinetics

With respect to the clinical pharmacokinetic data, no reports have been published showing the pharmacokinetic profile of elacridar alone. However, Kuppens et al. reported the pharmacokinetic profile for elacridar (dose ranging from 100 to 500 mg) upon co-administration with topotecan. No linear increase in the systemic exposure (either C_{max} or AUC) for elacridar was observed with 5-times escalation in the dose and the time to reach maximum plasma concentration (T_{max}) ranged from 4 to 6 h across the all the tested doses [53]. The observations drawn from the plasma concentration versus time profiles of elacridar suggested a long half-life value for elacridar although it was not reported [53]. As elacridar was dosed along with topotecan, it may be difficult to predict the actual pharmacokinetic behaviour of elacridar because the effect of topotecan on the pharmacokinetic profile of elacridar cannot be ignored. Elacridar potentially inhibited BCRP to a greater extent than compared to P-gp that has been evident from the biodistribution study in mice. Therefore, brain-to-plasma partition coefficient of elacridar in the wild-type mice, which was only 0.82, was dramatically enhanced to 3.5 in MDR1a/b(−/−) mice, 6.6 in BCRP1(−/−) mice and 15 in MDR1a/b(−/−)BCRP1(−/−) mice, indicating that both P-gp and BCRP limit the brain distribution of elacridar [52].

6 Preclinical Studies

6.1 Anticancer

Tang et al. observed that elacridar increased the brain accumulation of carbazitaxel by 9.6-fold in wild-type mice and the observed concentrations were similar to that observed for ABCB1a/1b;ABCG2−/− mice treated with or without elacridar. However, no significant enhancement in the plasma concentration of carbazitaxel was observed in the wild-type animals, which may be due to the higher selective brain uptake of carbazitaxel without altering systemic levels [54]. The brain-to-liver and brain-to-kidney concentrations were 10- to 14-fold higher in the presence of elacridar and were equivalent to that observed in both ABCB1-deficient strains. The findings of this study inferred that intravenous elacridar co-administration could completely inhibit the activity of mouse ABCB1 in the BBB, leading to highly increased cabazitaxel concentrations in the brain and, therefore, may translate into better efficacy [54]. Elacridar significantly increased the plasma concentrations of SN-38 (metabolite of topotecan) in wild-type and ABCC4, ABCB1 and ABCG2 knock-out mice suggesting that plasma level of SN-38 is mainly due to the inhibition of ABCG2-mediated elimination because ABCC4 + ABCB1 + ABCG2 knockout animals showed 2 times higher plasma concentration of SN-38 as compared to ABCC4 + ABCB1 knockout animals [55]. With respect to brain concentrations, the results suggested that only one of the aforementioned three genes is sufficient to maintain the similar brain concentration equivalent to wild-type animal's brain concentration of SN-38 [55]. Elacridar administration had no effect on the plasma concentration of gimatecan in wild-type animals which may be perhaps due to the sub-efficacious dose of elacridar to inhibit ABCB1a/b;ABCG2 significantly. However, a 1.5 and 3 times higher plasma concentration was observed for gimatecan in ABCB1a/b;ABCG2 knock-out animals at 1 and 4 h, respectively. A marginal increase in the brain concentration of gimatecan was observed in the wild-type and knock-out animals dosed with elacridar [55]. Elacridar increased the oral bioavailability of a campothecin analogue AR-67. Rats dosed with elacridar and AR-67 lactone orally resulted in 5.5- and 11-fold increase in the plasma lactone and carboxylate concentrations, respectively, by decreasing the clearance [56]. Similarly, animals dosed with AR-67 carboxylate resulted in 4.2- and 5.2-fold increase in the plasma lactone and caroboxylate concentrations, respectively [56]. Another study by de Vries et al. showed that elacridar increased the brain concentrations of topotecan in both wild-type, MDR1a/b(−/−) and BCRP1(−/−) knock-out mice but was not significant in MDR1a/b(−/−);BCRP1(−/−) knock-out mice suggesting that both MDR1a/b and BCRP1 are responsible for limiting the brain access of topotecan [57]. Elacridar increased the plasma concentrations of topotecan by six- and ninefold in P-gp deficient and wild-type mice by increasing the intestinal uptake and decreasing the clearance and hepatobiliary excretion [57]. The relative fetal penetration of topotecan was twofold higher in P-gp deficient mice as compared to vehicle control animals, suggesting a function for BCRP in the maternal–fetal barrier of the placenta [58]. The results of positron emission tomography (PET) study in mice using [^{11}C]-topotecan showed a twofold increase in the brain concentration of elacridar as compared to the group treated only with [^{11}C]-topotecan [59].

Kemper et al. described that elacridar increased the brain concentration of docetaxel by 59% in P-gp knock-out mice [60]. Elacridar increased the plasma concentration of paclitaxel by 3.8 times in wild-type mice [61]. Kemper et al. observed that elacridar increased brain concentration of paclitaxel by fivefold in P-gp knockout animals as compared to wild-type control animals [62]. Oral co-administration of elacridar increased the bioavailability of

paclitaxel and docetaxel by 10.7- and 4-fold in CYP3A4-humanised mice. Although the brain concentration of both paclitaxel and docetaxel increased in the presence of elacridar, the brain-to-plasma ratio remained unaffected [63].

Minocha et al. observed that elacridar increased the brain distribution of pazopanib by 2.1 times in FVB wild-type mice without affecting the plasma concentration. This may be due to the preferential uptake of pazopanib into the brain tissues and similar to what was observed for erlotinib and canertinib. No significant difference in the clearance and half-life of pazopanib was observed in the presence of elacridar. However, elacridar increased the volume of distribution of pazopanib by 1.4 times as compared to native pazopanib [64]. Elacridar increased the brain-to-plasma ratio of cobimetinib by 11, 6 and 7 times in MDR1a/b(−/−) and MDR1a/1b/BCRP1(−/−) knock-out and wild-type mice [65]. Tang et al. described that elacridar increased the plasma concentration and brain-to-plasma ratio of crizotinib by 2.2- and 12-fold in wild-type mice, respectively, compared with the vehicle-treated group and the concentrations were equivalent to that obtained from ABCB1a/1b;ABCG2(–/–) mice [66]. The brain levels of dasatinib increased by fivefold in wild-type mice in the presence of elacridar [67]. Another study by Lagas et al. showed a 2-time increase in the plasma concentration of dasatinib in wild-type mice in the presence of elacridar, but no change was observed in ABCB1a/1b;ABCG2−/− mice [68]. In the same study, the brain levels of dasatinib increased by 11- and 1.6-times in elacridar-treated wild-type and knock-out mice, respectively, as compared to the vehicle-treated counterpart [68]. Thus, the brain-to-plasma ratio of dasatinib increased by 2.3and 1.3-times in elacridar-treated wild-type and knock-out mice as compared to the vehicle-treated counterpart [68]. However, elacridar was not found effective in increasing the brain concentration of dasatinib in platelet-derived growth factor-B (PDGF-B)-driven brainstem glioma model in mice [69]. Co-treatment with elacridar increased the concentration of erlotinib in the tumour core by fourfold and by 14-fold in the regions around the brain as observed in a study on U87 rat xenograft model [70]. Radiolabelled pharmacokinetic study of [^{11}C]-erlotinib in the presence of elacridar showed a 5.3-time increase in the brain concentration in wild-type mice as compared to vehicle-treated group and the observed level was equivalent to that of ABCB1a/b(−/−)ABCG2(−/−) mice [71]. Elacridar increased the brain-to-plasma ratio of gefitinib by fourfold in wild-type mice [72]. Kawamura et al. described that the brain-to-blood ratio of [^{11}C]gefitinib increased by 4 and 11times following intravenous injection at 5 and 50 mg/kg dose levels, respectively [73]. Elacridar increased the blood concentration of imatinib in a dose-dependent manner where 1.8-fold increase was observed at a dose of 3 mg/kg and 4.4-fold at 30 mg/kg. However, the pattern was not same with respect to the brain-to-blood ratio [74]. The brain-to-blood ratio of animals treated with elacridar + imatinib showed ninefold higher value as compared to the animals treated with imatinib only [74]. Elacridar also increased the blood and brain concentration of the radioactive metabolites of imatinib by 1.7- and 2.8-fold, respectively, in Mdr1a/1b(−/−) mice [74]. Breedveld et al. demonstrated that elacridar increased the brain penetration of imatinib (following intravenous administration) by 4.2-fold and also reduced the clearance by 1.7-fold in wild-type mice [75]. Bihorel et al. described that elacridar increased the brain uptake of imatinib in wild-type (4.1-fold) and MDR1a/1b(−/−) mice (1.2-fold) [76]. Co-administration of elacridar increased the ($AUC_{0\text{-inf}}$) oral and ($AUC_{0\text{-inf}}$) IV by 3.3- and 2.0-fold, respectively, in wild-type and 2.7- and 1.3-fold in MDR1a/1b/BCRP1−/− mice. The percentage increase in oral bioavailability of imatinib when elacridar was co-administered was 105 and 102% in wild-type and MDR1a/1b/BCRP1−/− mice, respectively [77]. Elacridar also increased the brain concentration of sunitinib by 10 times in wild-type mice; however, no significant change in the plasma concentration was observed which may be due to the preferential uptake of sunitinib in the brain relative to increase plasma concentrations [78]. Tang et al. observed that elacridar increased the plasma con centration of *N*-desethyl sunitinib (active metabolite of sunitinib) by 1.4 times in wild-type mice. *N*-desethyl sunitinib was not detectable in the brain of wild-type mice in the absence of elacridar; however, 10 ng/g concentration of the metabolite was observed in the presence of elacridar [79]. Oberoi et al. described that brain-to-plasma ratio for sunitinib after co-administration of elacridar in wild-type mice was ∼12 compared with ∼17.3 in MDR1a/b(−/−)BCRP1(−/−) mice [80]. Elacridar was found to increase the brain concentration of palbociclib by 22 times in wild-type mice as compared to the group treated with only palbociclib; however, no significant change in plasma concentration was observed [81]. Although no significant enhancement in the plasma concentration of sorafenib was observed in the presence of elacridar, the brain concentration was 7 times higher in wild-type mice, which may be due to the preferential uptake of sorafenib in the brain [82]. Elacridar increased the brain-to-plasma ratio of sorafenib by 8 times in wild-type mice [83]. Minocha et al. observed that elacridar increased the brain-to-plasma ratio of vandetanib up to fivefold in FVB wild-type mice [84]. A 3- to 5-fold increase in the brain concentration of vemurafenib was observed upon co-treatment with elacridar [85].

6.2 Anti-retroviral

Edwards et al. observed 8 times increase in the brain-to-blood ratio of amprenavir in the presence of elacridar in

rats [86]. Similarly, a 100-fold increase in the brain-to-plasma ratio of nelfinavir was observed in the presence of elacridar in rats [87]. Elacridar-treated wild-type mice showed a significant increase in atazanavir C_{brain}/C_{plasma} (12.3-fold) and C_{testes}/C_{plasma} (13.5-fold) ratios compared to those in vehicle-treated counterparts [88]. Huisman et al. observed a 4.4-fold increase in the plasma concentration and tenfold increase in the brain concentration of saquinavir upon co-treatment with elacridar [89]. The oral bioavailability of ritonavir was increased by 2.7 times upon co-administration of elacridar in rats [90].

6.3 Miscellaneous

Elacridar increased the plasma concentration of convallatoxin (cardiovascular agent) by 1.5 times and by 2 times in the brain of in rats [91]. Zhang et al. observed that elacridar increased the brain-to-plasma ratio of EZH2 inhibitor that is used for the treatment of gliomas by 12-fold without affecting the plasma concentration [39]. The brain distribution of GSK2126458, phosphoinositide 3-kinase inhibitor, was enhanced by sevenfold in wild-type mice [92]. Elacridar increased the systemic exposure of GV196771, an N-methyl-D-aspartate receptor antagonist, by more than tenfold in wild-type mice [93]. The brain-to-plasma ratio of YQA-14, a novel dopamine D3 receptor antagonist, increased by more than 75-fold with co-administration of GF120918 in mice [94].

A twofold decrease in the biliary clearance was observed for acetaminophen sulfate in rats upon co-administration of elacridar [95]. Lee et al. observed a 2-times increase in the brain uptake of radiolabelled dehydroepiandrosterone in wild-type mice [96]. In the presence of elacridar, the ratio of brain to plasma ratio in mouse increased 2-, 4- and 38-fold, respectively, for talinolol, digoxin and quinidine, whereas in rat, a 70-fold increase was observed for quinidine [97]. Pre-treatment of mice infected with *Cryptococcus neoformans* with elacridar significantly increased the cerebral concentration of elacridar [98]. Elacridar increased the brain distribution of loperamide by 3.5-fold in rats [99]. Barraud de Lagerie et al. observed that elacridar increased the brain concentration of (+) and (−)-mefloquine by 2.5 and 1.5 times in mice without affecting the plasma concentration. The efflux clearance from the brain decreased for both enantiomers, with a larger decrease for (+)-mefloquine [100]. Elacridar did not alter the plasma levels of quinidine and verapamil in rats. However, there was a twofold increase in the plasma concentration of digoxin in the presence of elacridar. The brain and CSF levels of quinidine, verapamil and digoxin increased 21–26- and 6–11-fold, respectively [101]. Elacridar increased the brain tissue:serum concentration ratio of morphine by approximately threefold in rats [102]. The half-life of unbound morphine in brain extracellular fluid was approximately threefold longer in elacridar-treated rats compared with normal counterparts [102]. The fraction unbound of morphine in whole blood was not altered significantly in the presence of elacridar as compared with controls [102]. The CNS uptake of riluzole was increased by 3 times in mice, in the presence ofelacridar [103]. Elacridar increased the brain distribution of verapamil in rats by 11-fold [104].

7 Clinical Studies

Sparreboom et al. described that elacridar increased the systemic exposure of doxorubicinol (major metabolite of doxorubicin) following doxorubicin administration by 1.5 times at a dose level of 200 mg b.i.d (elacridar) and by more than 2 times at 400 mg b.i.d (elacridar) dose in cancer patients [105]. Planting et al. observed no linear increase in the response for doxorubicin with the escalation of elacridar dose from 50 to 100 mg b.i.d: however, linear increase in the plasma concentration was observed from 100 to 400 mg b.i.d in a clinical study in 46 cancer patients. Varying doses of doxorubicin (50, 60, 75 mg/m^2) had no impact on the pharmacokinetic profile of elacridar. Significant interpatient variability was observed in the pharmacokinetics of elacridar. The AUC of doxorubicin was only marginally influenced by elacridar and only at the highest dose level; however, elacridar significantly increased the systemic exposure of doxorubicinol [46]. Elacridar (Dose: 1000 mg) increased the oral bioavailability of paclitaxel by 5 times upon co-administration in cancer patients ($N = 6$) and was found to be well tolerated. AUC ratio for the metabolites 6α-hydroxypaclitaxel and 3′p-hydroxypaclitaxel was 1.1 (0.40/0.36) after oral drug administration with elacridar, whereas this ratio was 3.5 (1.69/0.48) when paclitaxel was combined with cyclosporine A which might be due to higher P-gp inhibition potential of paclitaxel at the tested dose as compared to elacridar [106]. The oral bioavailability of topotecan was found to be more than 102% higher in the presence of elacridar (Dose: 100 mg) administered concomitantly in 39 cancer patients. Two dose-limiting toxicities were seen at the 2.5 mg topotecan dose level [53].

8 Discussion

The choice of elacridar for this review compilation stemmed from the fact that elacridar has been extensively studied amongst all other reported P-gp inhibitors which are in development. More importantly, the observations and conjectures drawn using elacridar as an example can be

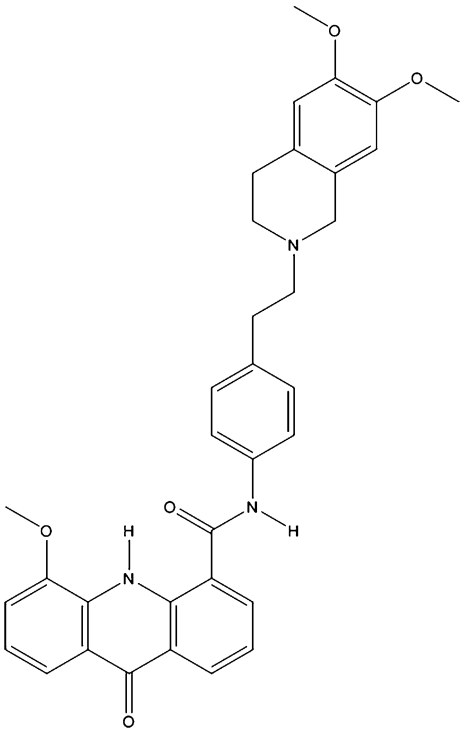

Fig. 1 Chemical structure of elacridar

extrapolated to the whole class despite differences in the pharmacokinetic properties which may be compensated by appropriate dose sizes.

Plethora of in vitro and in vivo data reviewed in this paper provides the basis for an effective blockade of P-gp efflux mechanism by elacridar. The beneficial effect of such a blockade if successfully translated in clinical practice would revolutionize many therapeutic areas most notably in the oncology area based on the huge body of collective evidence for various chemotherapeutic drugs Fig. 1.

An important introspection worth considering is why drugs of this class including elacridar with so much promise and potential have not reached the stage of market approval yet. In this context, elacridar along with other P-gp inhibitor drugs in this class provides a distinct advantage as compared to its predecessors in not having cytochrome P450-related inhibition liability. However, despite such a differential feature in its disposition, it appeared that there was still lacuna in the complete understanding of key requirements when elacridar and/or other P-gp inhibitors are given in a combination therapy [107]. To address this important question, it is necessary to closely examine the ADME characteristics of elacridar along with other drugs of this class (Table 4); unfortunately, the pharmacokinetic reports for the listed drugs are scanty. It appeared that elimination half-life for several P-gp inhibitors including elacridar were relatively long (>18 h); however, it would be uncertain as to what this would mean at the tissue level of P-gp inhibitors. Therefore, would once-a-day dosing for P-gp inhibitors be justified is an important question that needs to be addressed. As displayed in Fig. 2, the key ADME points of interaction by elacridar which may be representative of the entire class would likely incorporate multiple non-specific interactions of the efflux mechanisms from the site of absorption to various distribution sites including brain, liver, kidney, etc. Also, the excretory mechanisms such as biliary and/or renal may also be affected by elacridar. Interestingly, there may be altered entero-hepatic recycling phenomenon because of elacridar on the indigenous transporters both at enterocytes and biliary efflux transporters. To underscore the above views, Srinivas has suggested that there was a need to understand the possible interplay of various mechanisms of a P-gp inhibitor drug when co-administered with cytotoxic drugs using tariquidar as an example [22]. For instance, if the P-gp inhibitor has an inhibitory role in the biliary excretion of the cytotoxic drug, the co-administration would result in an increased systemic exposure of the cytotoxic drug. However, if the same P-gp inhibitor simultaneously decreases efflux within tumour cells, it may promote greater accumulation of the cytotoxic drug within the tumour environment. Therefore, the net effect on systemic exposure may be neutral although the excretory pathway of the cytotoxic drug was impacted by the P-gp inhibitor.

There are a number of developmental considerations that need to be strategized for ensuring that optimal delivery of elacridar and/or other members of this class occur in the

Table 4 Pharmacokinetic parameters of third-generation P-gp inhibitors in human clinical studies

P-gp inhibitors (reference)	Dose (route of administration)	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg·h/mL)	CL or CL/F (L/h)	V_d (L)
Tariquidar [114]	8 mg/kg (IV)	1.62 ± 0.35	2.42	18.06 ± 5.89	19.13 ± 3.62	17.87 ± 3.18	527 ± 116.88
Zosuquidar [18]	480 mg/m^2 (IV infusion)	0.43			19.40		
Laniquidar [115]	200 mg (oral)	0.12 ± 0.06		19.6 ± 7.4	0.55 ± 0.24		
ONT-093 [116]	300 mg (oral)	1.32 ± 1.13			6.62 ± 5.29		

Data expressed as mean ± SD (wherever reported), except for t_{max} which are expressed as median

AUC area under the curve, *CL/F* total body clearance, C_{max} maximum plasma concentration, *IV* intravenous, $t_{1/2}$ half-life, V_d volume of distribution

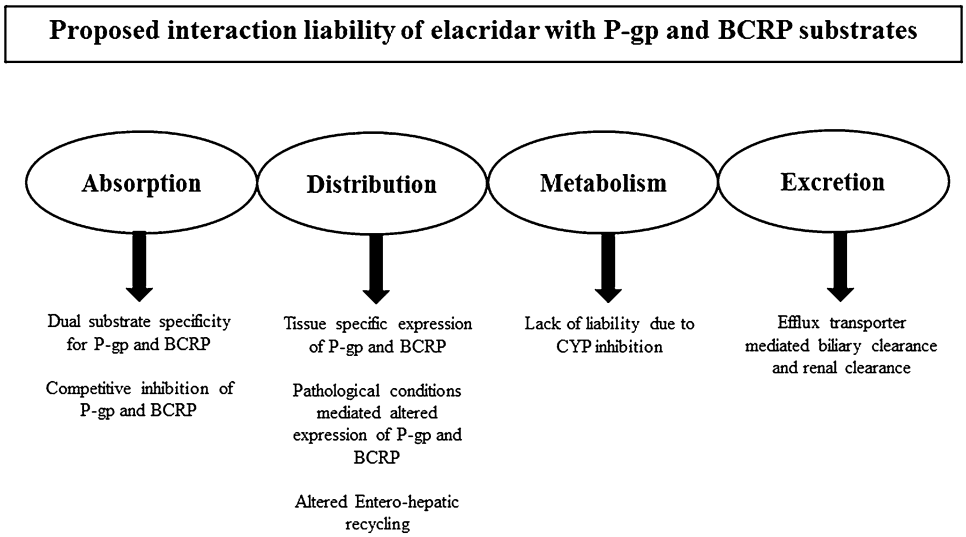

Fig. 2 Representation of interaction liability of elacridar during absorption, distribution, metabolism and excretion (ADME) process. P-gp permeability-glycoprotein, *BCRP* breast cancer resistance protein, *CYP* cytochrome P450

patient population. First, do elacridar and/or other P-gp inhibitors of this class reach the purported site of action at the threshold concentration to elicit the efflux inhibitory response? Because in both clinical studies elacridar was orally administered, the issue of absorption and oral bioavailability is not an important consideration. One important question would be whether increasing oral doses of elacridar have an impact on its own rate and extent of absorption and, therefore, the ability of elacridar to reach the purported site of action would be expected to be highly variable. Similar issues would also prevail with other P-gp inhibitors in this class such as tariquidar, zosuquidar, laniquidar, etc. Besides debating on the lack of effectiveness of oral P-gp inhibitors, the lack of convincing clinical data on the effectiveness of P-gp inhibitors even after giving intravenous doses does raise a concern. Second, few other questions such as (a) adequacy of intravenous dose size of the P-gp inhibitor drug, (b) choice of the right regimen/schedule of the P-gp inhibitor (whether it should closely mimic the chosen cytotoxic drug), (c) distribution characteristics and elimination kinetics of the chosen P-gp inhibitor in relation to the cytotoxic drug need to be probed to possibly to ascertain areas of concern for remedial measures, if any. Third, what are the inadvertent consequences of lack of specificity of elacridar and/or P-gp inhibitor drugs? Because of extensive tissue distribution of such P-gp inhibitor drugs (i.e., the distribution volume of tariquidar is >100 times the total blood volume), it may be possible that it may promote accumulation of cytotoxic drug(s) in another area (i.e., brain, kidney, intestine, etc.) leading to undesired adverse reactions or side effects. However, from a benefit:risk analysis, the greater good of tumour shrinkage should outweigh such episodes of adverse events and/or safety issues if they not considered life-threatening or fatal. Fourth, because of non-specific nature of P-gp inhibition, it should be generally expected that elacridar and/or other P-gp inhibitors belonging to this class, should also impede the excretion mechanisms of the co-administered cytotoxic drugs (Fig. 2). Therefore, either reduced clearance or biliary excretion of cytotoxic drugs should only promote increased systemic circulation of cytotoxic drugs. While in theory, a more sustained concentration of cytotoxic drug is to be expected in circulation in presence of elacridar and/or other P-gp inhibitor drugs, and this in turn should provide a reservoir effect to promote increased uptake of cytotoxic drugs into the tumour tissues where P-gp efflux mechanisms would be expected to be turned off. But the only caveat would be how to measure the transfer of cytotoxic drug from the systemic circulation to tumour tissues whether it is primary tumour or metastasized tumour sites. However, with the availability of current day modelling tools including physiology based pharmacokinetic modelling it may be possible to try to answer this question. Fifthly, it may be equally important to match the pharmacokinetics of the cytotoxic drug (distribution, excretory mechanisms and half-life) with the chosen pharmacokinetic attribute of the P-gp inhibitor to ensure that an overlapping synergy exists to maximize the therapeutic effectiveness. Therefore, one important realization should be that not all cytotoxic drugs may be targeted with the same dose size of elacridar and/or other P-gp inhibitors and there would be a clear need for the optimization of the P-gp inhibitor dose based on cytotoxic drug(s) and perhaps, the type of tumours being targeted.

Similar scenario of interactions of small molecule tyrosine kinase inhibitors used in cancer therapy with P-gp inhibitors is likely to manifest during absorption, distribution and excretion of such tyrosine kinase inhibitors

[108]. However, because several tyrosine kinase inhibitors may also affect efflux or uptake transporters, the net consequences of co-administration of tyrosine kinase inhibitors with P-gp inhibitors is difficult to predict. Also, since cancer treatment is very much a combination therapy that may include cytotoxic drug(s) along with tyrosine kinase inhibitors, the use of P-gp inhibitor need to be made with caution to avoid dangerous drug–drug interaction potential which may be difficult to predict a priori using in vitro tools and/or in silico models.

P-gp inhibition and the relevance to antidepressant therapy have been a topic of great interest because of the opportunity to deliver antidepressant drugs effectively to brain overcoming the P-gp based efflux mechanism. A review published several years ago has provided a critical assessment on issues pertaining to evolution of the field of antidepressant therapy with P-gp inhibitors [109]. While P-pg inhibitors may provide an opportunity to increase the brain penetration of antidepressants for treating refractory patients, the translatability in the clinic is yet to be established in an unambiguous manner [109].

From all the above viewpoints, it is important that both study design (pre-treatment with P-gp inhibitor *versus* simultaneous dosing) and dose size (due to differences in excretory mechanisms for P-gp inhibitors relative to cytotoxic drugs) be given careful consideration. To underscore the above point, biodistribution of the labelled tariquidar suggested relatively higher uptake of the drug in liver, spleen and kidneys in humans and, therefore, the importance of excretory pathways such as hepatobiliary and renal to rapidly remove P-gp inhibitor drug such as tariquidar from circulation should be factored in both study design and dosing decisions [110].

9 Conclusions

Innovative approaches to counter efflux mechanisms via P-gp inhibitors have been tried using third-generation P-gp inhibitors such as elacridar, tariquidar, zosuquidar, laniquidar, etc. While the promise demonstrated by scores of in vitro and preclinical in vivo data of elacridar and other drugs in the class was encouraging, unfortunately the translatability in a desired clinical outcome is yet to be established in oncology trials where P-gp inhibitors are given in combination with cytotoxic drugs. There are number of critical questions that need to be considered while attempting to understand and solve this problem. Key developmental considerations would encompass the following: (a) whether P-gp inhibitors be pre-treated as opposed to given simultaneously with the coadministered drug; (b) as to how should one choose the dose size and schedule; (c) as to what are the overlapping ADME mechanisms that need to be considered to assess the drug interaction potential; (d) whether plasma concentration (i.e., threshold level) of P-gp inhibitor drug be used as a surrogate from a dosing strategy perspective. It appears that a thorough understanding of the interplay of various mechanisms is a key for the successful development of P-gp inhibitor drugs in assessing the potential risks versus therapeutic benefits in combination therapies. In summary, the clinical translation into a desirable outcome has still been elusive for P-gp inhibitor drugs not only in cancer therapeutics but also in other areas; however, the promise of avoidance of efflux mechanisms in desired regions would revolutionize current treatment options in multiple therapeutic areas.

Compliance with Ethical Standards

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