Be Careful What You Ask for: Challenges of Predicting Human Clearance for a Low Metabolic Turnover Compound, ELND006

Kevin Quinn[1], David Nakamura[1], Heather Zhang[1], Shawn Gauby[1], Colin Lorentzen[1], Erich Goldbach[1], Amanda Moore[2], Salman Khetani[2], Earvin Liang[3], John-Michael Sauer[1] and George Tonn[1],
[1]Drug Metabolism and Pharmacokinetics, Elan Pharmaceuticals, South San Francisco, CA, [2]Research and Development, Hepregen Corporation, Medford, MA,
[3]Clinical Development, Elan Pharmaceuticals, South San Francisco, CA

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Introduction

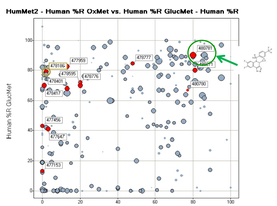
The discovery of a new chemical entity (NCE) suitable for CNS drug development is a challenging endeavor as many different properties require simultaneous optimization. One of these properties, metabolic stability, is a surrogate for *in vivo* clearance (CL). A common practice of discovery project teams is to select NCEs with the greatest metabolic stability for further evaluation. With NCEs that exhibit low or no turnover in *in vitro* systems, it becomes very challenging to project human CL and thereby the plasma profile of the compound (i.e., half-life; $T_{1/2}$). Compounds with excessively long $T_{1/2}$ can pose development challenges (e.g., long washout times for cross-over study designs, necessitating loading doses for rapid onset of activity, ensuring an adequate duration of exposure coverage from safety studies, etc.). While these are not "show stoppers", *a priori* projections of pharmacokinetic (PK) properties of these NCEs does provide a basis for the rationale design of early clinical development programs prior to the availability of human data. In addition, very low turnover of an NCE complicates the characterization of metabolites. One criterion for the selection of non-rodent species for safety assessment is the similarity of its metabolite profile to humans.

ELND006 (Table 1), a potent and APP selective gamma secretase inhibitor that lowers Aβ in the CNS of rodents and nonhuman primates[1] was selected from a series of NCEs based, in part, on its metabolic stability in both NADPH and UDPGA supplemented liver microsomes (Figure 1).

Table 1: Selected *In Vitro* Properties of ELND006

ELND006	APP IC_{50} nM	Cell APP/Notch Selectivity	LogD	Solubility µM	Permeability nm/s	P-gp Efflux BA/AB	Protein Binding $F_{u,plasma}$	$F_{u,microsomes}$
	0.3	82	3.91	35	76	1	0.005	0.06

Figure 1: Oxidative (X-Axis) and Phase II (glucuronidation; Y-Axis) Metabolic Stability of Gamma Secretase NCEs in human liver microsomes. ELND006 is in the quadrant with the greatest metabolic stability.



Legend: plot of single time-point (30 min) stability screens for test articles (1 µM) incubated with human liver microsomes (0.5 mg/mL) supplemented with UDP- glucuronic acid (y-axis) or NADPH (x-axis). Symbols are sized by the inverse of their potency to inhibit Gamma APP cleavage. Selected analogs are highlighted with ELND006 (aka, ELN 480781) in the quadrant of greatest stability (>80% of parent remaining)

This high metabolic stability proved challenging for ELND006 since human CL could not be predicted based on *in vitro* data and the low turnover generated only trace levels of metabolites making identification of them difficult. Ultimately, ELND006 was evaluated in clinical studies. These studies showed that ELND006 exhibited very low CL (0.054 L/h/kg) following oral administration, resulting in a very long half-life (220 to 306 hours)[11].

Objectives

1. Retrospectively evaluate allometry, microsomes, suspended hepatocytes and micropatterned co-cultures of hepatocytes and fibroblasts (HepatoPac™) to estimate human CL of ELND006.
2. Characterize the *in vitro* metabolic profiles of ELND006 generated with the HepatoPac system.

Methods

***In Vitro* Methods**:
The turnover of ELND006 was evaluated in both human liver microsomes (HLM) and suspended human hepatocytes. Briefly, ELND006 was incubated at 1 µM in pooled HLMs (0.5 mg/mL, BD Gentest, Woburn MA) and NADPH (1 mM) for 45 min at 37°C. The compound was also incubated with cryopreserved human hepatocytes ($1x10^6$ cell/well, BD Gentest, Woburn MA) from multiple donors in suspension over 4 hours. Serial samples were collected at various time points by quenching the reaction with the addition of acetonitrile. Samples were centrifuged and the supernatant was analyzed using a LC-MS/MS method (API-5000; ABI-Sciex, Foster City, CA). The disappearance of ELND006 was monitored.

Additional studies were conducted using micropatterned plated hepatocytes (HepatoPac System, Hepregen Corporation, Medford, MA). In these studies, 1 µM of ELND006 was incubated with cryopreserved rat and human hepatocytes (4500 cells/well in 96-well format) over seven days.[2] Samples were centrifuged and the supernatant was analyzed using a LC-MS/MS method (as above). The disappearance of ELND006 was monitored as well as the formation of characterized metabolites from specific mass transitions.

In vitro intrinsic clearance and hepatic clearance were calculated by the well stirred model incorporating both plasma and microsomal protein binding.[3,4] Key assumptions were that liver is the major organ of clearance and blood partition is negligible. The latter assumption is based on the fact that the blood to plasma partitioning of ^{14}C-ELND006 was approximately one in rats and monkeys (data not shown). Physiological parameters were based on literature estimates.[5] Literature values were used for systemic clearance and plasma protein binding for quinidine and warfarin[10], as well as microsomal and hepatocyte binding.[6]

Allometric Scaling:
Human clearance and volume of distribution parameters was estimated using allometric scaling with preclinical PK data obtained from mouse, rat, dog and monkey. Animals were dosed with ELND006 intravenously at 1 mg/kg or orally from 1 to 5 mg/kg. Samples were analyzed using LC-MS/MS. Non-compartmental PK parameters were calculated with WinNonlin (v 5.2, Pharsight Corporation).[7] Allometric scaling to human PK parameters was conducted based on a traditional approach and the rule of exponents following corrections for protein binding.[8,9]

Metabolite ID
^{14}C- ELND006 (10 µM) was incubated at 1 mg/mL HLMs for 60 min and with hepatocytes ($1x10^6$ cells/well) for 4 hours. Reactions were quenched with the addition of acetonitrile. The resulting supernatants were quantitatively analyzed with a 610TR radiochemical detector (Perkin-Elmer, Waltham MA) inline with an API-4000Qtrap (ABI-Sciex) or OrbiTrap (Thermo, San Jose CA). Plasma, blood, urine, feces and bile (rat and monkey) were collected following the IV administration of 1 mg/kg (75 µC/kg) ^{14}C-ELND006 dose. Samples were analyzed as described above. Studies utilizing^{14}C ELND006 were conducted at Covance Laboratories (Madison, WI).

Although HepatoPac experimental conditions were chosen for metabolic stability rather than metabolic identification (e.g., 30k cells/well in 24-well format), metabolite profiles in human HepatoPac supernatant and cell lysate samples were monitored using MS/MS transitions of charactrized metabolites determined in the ^{14}C studies.

Results

In Vitro
Attempts to calculate intrinsic clearance from *in vitro* studies were unsuccessful due to poor turnover of ELND006 in microsomes or hepatocytes (data not shown).

Plated micropatterned hepatocytes (HepatoPac system) were evaluated retrospectively to assess whether they could discriminate low metabolic turnover compounds better than traditional *in vitro* systems for a prediction of intrinsic clearance. The results for ELND006 and two other low turnover compounds, warfarin and quinidine, are reported in Table 2 for rat and human with their concentration-time data presented in Figure 2.

Figure 2: Concentration disappearance-time data of ELND006 (A, left panel), quinidine (B, center panel), and warfarin (C, right panel) in the supernatant following incubations in plated micropatterned human hepatocytes. Data for rat heptocytes not shown.

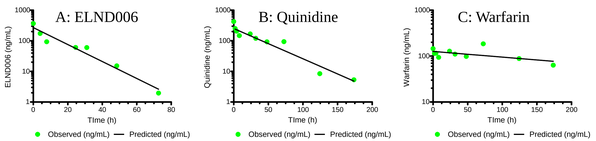


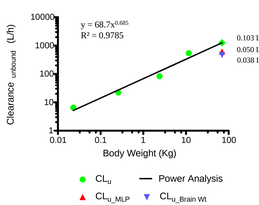
Table 2: Predicted *in vivo* clearance values from plated micropatterned hepatocytes.

Compound	Species	$T_{1/2}$ h	CL^A L/h/kg	CL^B L/h/kg	Literature L/h/kg	CL/F L/h/kg	Fold error (*in vitro* over-prediction) No binding correction	Fold error (*in vitro* over-prediction) Binding correction
ELND006	**Human**	**11.5**	**2.05**	**0.17**	**--**	**0.054**	**38-fold**	**3.1-fold**
	Rat	**4.17**	**5.67**	**1.14**	**1.02[a]**	**--**	**5.6-fold**	**1.1-fold**
Quinidine	**Human**	**30.4**	**0.78**	**0.13**	**0.28[10]**	**--**	**2.8-fold**	**0.46-fold**
	Rat	**6.06**	**3.90**	**--**	**1.9[12]**	**--**	**2.1-fold**	**--**
Warfarin (R/S)	**Human**	**243**	**0.10**	**0.001**	**0.003[10]**	**--**	**33-fold**	**0.3-fold**
	Rat	**270**	**0.09**	**--**	**0.01[13]**	**--**	**9-fold**	**--**

A – CL calculated without protein binding corrections
B – CL calculated using plasma protein binding corrections and estimated binding in hepatocytes based on microsomal binding
[a]Elan data on file

Allometry
Unbound clearance was empirically scaled using simple allometry (CL_u) and allometry using maximum life span potential (CL_{u_MLP}) and brain weight ($CL_{u\_Brain\ Wt}$) correction factors. The predicted human CL values ranged from 0.038 (brain weight correction, $CL_{u\_Brain\ Wt}$) to 0.103 L/h/kg (simple allometry, CL_u) as shown in Figure 3. However, using rule of exponents, simple allometry was chosen over other methods. Total volume of distribution was also scaled using allometry (not shown) and was estimated to be 5 L/kg. Based on these values, human half-life was estimated to range from 38 hours to 99 hours.

Figure 3: ELND006 Allometric scaling of human unbound clearance



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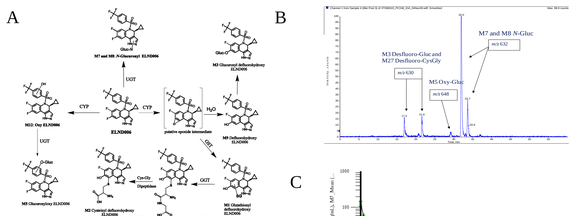
Metabolite Identification
The *in vitro* metabolite identification data collected using ^{14}C-ELND006 in microsomes and hepatocytes demonstrated the greatest similarity between monkey and human both qualitatively and quantitatively. The major metabolic pathway was via *N*-glucuronide formation (data not shown). The proposed metabolic pathway in human and monkey based on *in vitro* data is shown in Figure 4A. In the monkey, this was confirmed *in vivo* following ^{14}C-ELND006 IV administration to bile duct cannulated monkeys (Figure 4B). Of the 80% of the dose excreted in bile, the bulk of the radioactivity was excreted as two *N*-glucuronides (M7 and M8) followed by glucuronide conjugates of oxidative metabolites (M3 and M5) and a glutathione catabolite (M27).

Figure 4: Biotransformation pathway of ELND006 based on cumulative data (microsomes, hepatocytes and *in vivo* animal data – Panel A), *in vivo* metabolites of ^{14}C-ELND006 in monkey bile (Panel B) and qualitative evaluation of metabolites formed with the human HepatoPac System (Panel C).



Qualitative assessment using MS/MS transitions of known metabolites produced in plated human hepatocytes (HepatocPac System) demonstrated good agreement with prior *in vitro* data and data generated *in vivo* in the cynomolgus monkey (Figure 4C).

Discussion

Predictions of clearance for very low turnover compounds using *in vitro* systems can be challenging if not almost impossible as exemplified by results with ELND006. While it was evident from these data that ELND006 would exhibit low CL, the actual magnitude of the CL could not be accurately determined *in vitro*. Due to the high microsomal binding (Table 1), it is unlikely that increased microsomal protein or cell numbers would have had any benefit. In the absence of reliable *in vitro* data, human CL was estimated using allometry, which led to a half-life prediction six to eight-fold lower than that observed.

Micropatterned co-cultures of hepatocytes and fibroblasts (HepatoPac) allows for *in vitro* incubations for up to seven days (Khetani and Bhatia). Incubation of ELND006 in this system resulted in turnover of parent and formation key metabolites (Figures 2A and 4C). The prediction of human CL for warfarin and quinidine agreed well with literature reports when corrected for protein binding (Table 2). In the absence of protein binding corrections (plasma and microsomal), CL values were markedly over predicted for all compounds including ELND006. Protein binding corrections improved the prediction of ELND006, but the CL was still over predicted (on the same magnitude as allometry).
Austin *et al*. (2002) showed that varying the amount of microsomal protein can influence turnover[14]. In this analysis we used microsomal/hepatocyte binding as a surrogate for binding in the system. For the control compounds, warfarin and quinidine, under estimating the binding in the HepatoPac system would have minimal impact on the CL predictions since these compounds exhibit very low microsomal/hepatocyte binding (0-7% warfarin and 32-60% quinidine)[6]. Since ELND006 exhibits much higher microsomal binding (94%), any underestimation will have a much more profound impact on CL projections (Figure 5).

Our data suggests that while further investigations (in particular protein binding) are warranted, the HepatoPac system may offer an alternative to empirical methods for predicting the human CL of low turnover compounds such as ELND006.

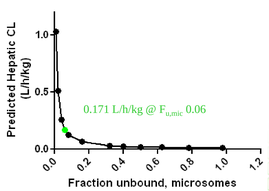


Figure 5: Sensitivity analysis of CL predictions for ELND006 vs. fraction unbound estimates.