Tropical Journal of Pharmaceutical Research April 2017; 16 (4): 911-917
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v16i4.24

Original Research Article

Fatal France clinical trial and the lessons learned: Application of in silico approaches to investigate the disposition of B1A10-2474 and possible safety concerns

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Received: 19 October 2016 *Revised accepted: 9 March 2017*

Abstract

Purpose: *Recent France clinical trial with the drug coded, B1A10-2474, turned out to be a safety failure due to the death of a participant and several adverse events involving other participants who were on multiple dosing. An attempt was made in this study to investigate if any possible caution could have been detected by early predictions by in silico methods.*
Methods: *The physiochemical properties of B1A10-2474 were obtained using ADMET™ predictors which were further inputted into SimCYP™ simulator to investigate the drug disposition in healthy subjects.*
Results: *B1A10-2474 had linear pharmacokinetics, tendency to accumulate, follow multiple compartment dispositions with a delayed phase of elimination, and high brain permeability with a linear relation with blood plasma concentrations.*
Conclusion: *Due to high brain permeability and possible accumulation in brain with multiple dosing, B1A10-2474 is a high alert drug. In silico approaches utilized in this study generated a safety caution for B1A10-2474 and hence these tools can be used in early drug development processes.*

Keywords: *B1A10-2474, French trial, SimCYP, PBPK, ADMET, Phoenix NLME*

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INTRODUCTION

Clinical trials are important stages for new drug candidates before they are made available to the larger human populations [1]. This in itself is one of the safety step undertaken to avoid the exposure of the drug to masses before safety of the drug is confirmed. The first human subjects to get the drug are often healthy subject as the standard practice in Phase I clinical trials.

Drugs are not free from side effects and some caution always been advised in using them effectively, however some of the milestone experiences with drugs have taught us lessons after incurring irreparable lose to the human race. Thalidomide tragedy was one of them which can never be forgotten. However these kind of tragedies put an extra pressure on the pharmaceutical companies to raise the safety standards with every bitter experience during the drug development process.

In recent years there have been tremendous improvement in the science and the process of drug development. Use of special software now allow us to predict the possible drug targets with as little information as the structure of the compound [1]. However, pharmaceutical companies usually make tremendous effort to get as

much details as possible from *in silico* predictions, *in vitro* experiments, *in vivo* studies in animal and experiments in human tissues on the safety and efficacy of the drug before administering the drug to humans. Despite all these efforts, drugs often surprise us upon first time exposure in humans either due to poor efficacy or safety issues. Moreover each drug poses a unique challenge, as drug development scientists we learn from these examples and exercise caution with future drug candidates.

Regulatory agencies while approving a clinical trial protocol rely on the document and experimental data presented by pharmaceutical companies which in the case of a missing otherwise crucial data could leave an important gap. On the other hand in house *in silico* simulations performed by regulatory agencies might guide them to demand for specific safety and efficacy data from the pharmaceutical companies in the interest of patients' safety.

Here we investigate the possible reason for the fatality of a phase I clinical trial [2,3] with as little information as the chemical structural of the Drug. The clinical trial in question claimed one human life with the drug candidate popularly known as BIA 10-2474. Use of *in silico* approaches to predict disposition of BIA 10-2474 and hint for any safety concerns, can serve as an example for application of *in silico* methods in drug development processes. Such approaches can be utilized to minimize harm to those healthy subjects who risk their lives and dare to consume the so called "investigational new drug (IND)" with limited safety information in humans use.

A major advantage of the *in silico* methods is that minimal information like the molecular structure is all that is required to predict the drug disposition and its targets, which can further be optimized upon the availability of the specific experimental data.

METHODS

Structure of the compound in the ".mol" file format was obtained from Chemspider (http://www.chemspider.com) bearing Chem-Spider ID: 41628677 [5]. Obtained structure was inputted into the ADMET predictor Version 7.2 (Simulations Plus, California, USA) to predict the physiochemical properties of the drug candidate. Further physiologically based pharmacokinetic (PBPK) modeling approach was used to predict the drug disposition in plasma and in brain tissues based on the physio-chemical properties obtained earlier. SimCYP simulator V 14.0.93 (SimCYP Limited, Sheffield, UK) was utilized to predict the absorption, distribution, metabolism and excretion (ADME) of different single and multiple doses of BIA10-2474 in healthy Caucasian male subjects. The compound file for BIA10-2474 was created in SimCYP based on the characteristic of BIA 10-2474 and the SimCYP inbuilt population characteristics for healthy Caucasians titled "Sim Healthy Volunteers) were used as physiological system data to study drug disposition.

PBPK modeling

SimCYP Advance Dissolution Absorption and Metabolism (ADAM) absorption model was utilized to predict the absorption upon oral administration of BIA 10-2474 and full-PBPK model for distribution, method 2 as described by Rodgers *et al* [4], was used to predict the volume of distribution. PBPK modeling was also used to predict the tissue concentration in brain tissues at different doses and automatic sensitively analysis (ASA) was performed for brain tissue partition coefficient concentration over area under the plasma concentration to investigate the possible drug accumulation in brain tissues. Pharmacokinetic simulations were performed for both single and multiple doses of the BIA 10-2474 mimicking the dose scenarios indicated in the clinical trial protocol for BIA 10-2474(5). Single doses simulations were performed for 0.25, 1.25, 2.5, 5, 10, 20, 40 and 100 mg doses and the sampling for pharmacokinetic evaluations were set to "pre-defined samples" until 72 h post dosing. Multiple doses simulations were performed for 10, 40 and 100 mg respectively given at an interval of 24 h for a total of ten doses and sampling for pharmacokinetic evaluations were set to "pre-defined samples" until 72 h of the last dose.

Population pharmacokinetic (POPPK) analysis

POPPK analysis was performed with Phoenix NLME (Certara, Princeton, NJ 08540, USA) for single dose of 40 mg of BIA 10-2474 obtained from the simulated concentration-time data for healthy subjects through PBPK modeling using SimCYP simulator. Initial pharmacokinetic parameters were obtained from Naïve pool analysis by WinNonlin 6.4 (Certara, Princeton, NJ 08540, USA) from the raw data. Observed data was fitted to build a base model followed by incorporating error model and covariates search to account for inter-subject variability. Stepwise additional or deletion of covariates, age, weight, brain weight and renal function on to the structural model was tested for statistical significant change in -2LL (-2*log likelyhood

ratio). Drop in -2LL of "-6.635" with addition or deletion of a covariate deemed statistically significant. Final model was then tested for precision by bootstrapping with 100 samples each with 1000 iterations. Finally model qualification was done by predictive checks with 1000 replicates and the parameter estimation with 5 and 95 % quintile obtained.

Different *in silico* approaches been adopted to drive the required data. Figure 1, represents the flowchart of the hierarchy of *in silico* approaches utilized in this study.

RESULTS

Physiochemical characteristics and liver stability of B1A10-2474

Physio-chemical properties and some liver stability data of B1A10-2474 predicted by ADMET predictor are summarized in Table 1 and Table 2 respectively. Predictions results suggest that B1A10-2474 has high blood brain barrier permeability with good intrinsic water solubility. In terms of the enzymes responsible for the metabolism B1A10-2474, simulation results predicted that it is metabolized by CYP1A2 and CYP3A4 cytochrome P40 enzymes. ADMET predictor also cautioned a qualitative report of possible CYP3A4 inhibition potential by B1A10-2474, however due to lack of quantitative assessment this was not incorporated in PBPK modeling to predict drug disposition.

Simulated pharmacokinetics with SimCYP

Concentration-time profiles of the different single doses of B1A10-2474 are presented in Figure 2. Ratio of plasma area under the curve to dose for all doses tested was "0.74", suggesting B1A10-2474 for doses ranging from 0.25 to 100 mg follows linear pharmacokinetics.

Table 1: B1A10-2474 specific parameters obtained from ADMET predictor

Parameter (*units*)	Value
Molecular weight^	300.45
P_{ka}^	3.63
LogP^	0.56
P_{eff}^ (*cm/s*10^4*)	1.7
Intrinsic water solubility^ (*mg/mL*)	7.63
BBB filter*	High
Unbound blood to plasma protein^ (*%*)	41.08
Blood to plasma concentration ratio^	0.95
fu_{mic}^	0.916

*Pe_{ff}- human jejuna effective permeability; fum_{ic}-fraction unbound in human liver microsomes; BBB-blood brain barrier; *qualitative prediction of penetration to brain tissue; ^parameter value utilized in simulations of pharmacokinetics of B1A10-2474 with SimCYP simulator*

Table 2: B1A10-2474 liver stability parameters predicted by ADMET predictor

Parameter (units)	*CYP1A2*	*CYP3A4*
Km^ (µM)	100	184
Vmax^ (nmol/min/nmol)	86.2	17.5
Clint (µL/min/mg)	44.7	11.1

Km = Michaelis-Menten constant; Vmax = maximum velocity; Clint = Intrinsic clearance for respective enzymes for which B1A10-2474 is a substrate; ^Parameter value utilized in simulations of pharmacokinetics B1A10-2474 with SimCYP simulator

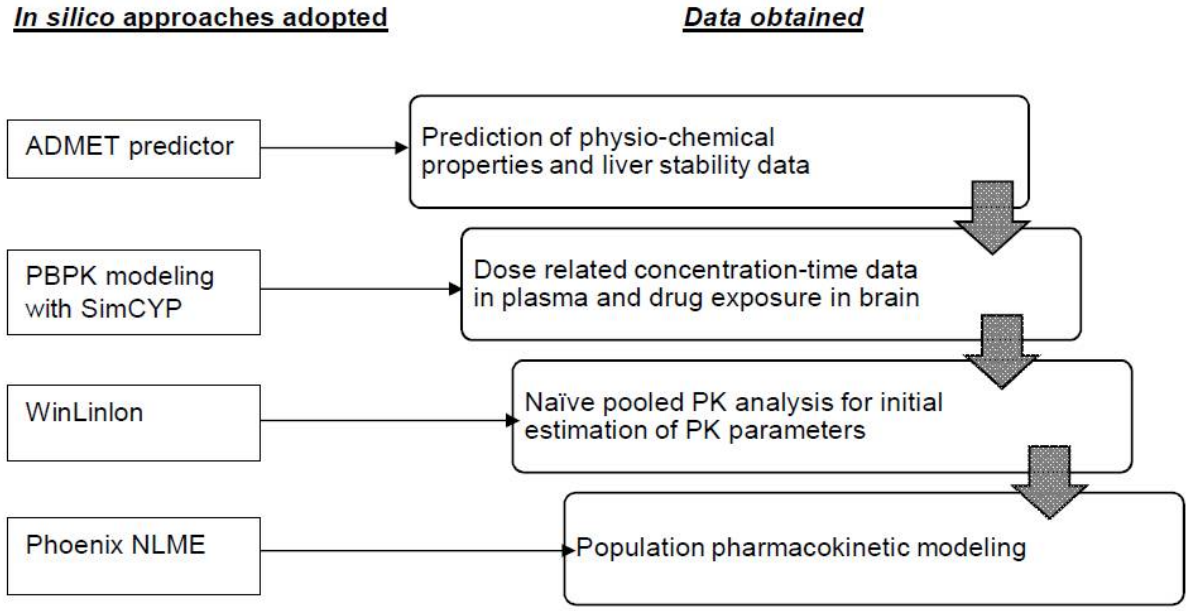

Figure 1: Hierarchy of *in silico* approaches adopted and utilization of obtained data in subsequent steps

The mean natural log transformed plasma concentration time profile for 40 mg of B1A10-2474 in healthy male subjects (Figure 2 (b)) suggest that B1A10-2474 pharmacokinetics can be best described by multiple compartment pharmacokinetic model which was further confirmed by POPPK analysis using Non-linear mixed effects. Sensitive analysis for dose range 0.25-100 mg with 10 steps over clearance and volume of distribution showed no dependence of dose on clearance and volume of distribution. Automatic sensitive analysis of brain tissue partition coefficient for range of 0.05 to 4.6 with 5 steps over plasma suggested drug exposure to brain tissue is in linear relation with the plasma area under the curve suggesting increased brain exposure with raised exposure of drug in plasma for all the single doses studied (Figure 3).

Plasma concentration profiles of multiple dose simulations for 10, 40 and 100 mg given at the interval of 24 h for ten doses are presented in Figure 4. ADMET predictor data indicated likely CYP3A4 inhibition potential of B1A10-2474, however due to lack of quantitative assessment this was not included in the SimCYP PBPK modeling. CYP3A4 auto inhibition would significantly alter the drug disposition in multiple dosing scenario, such situations could be predicted with SimCYP simulator provided qualitative data is available.

POPPK of 40 mg single dose

Initial parameters for Ka, Vd and Cl, 0.054/h; 0.25/L and 0.015 L/h, respectively, obtained from naïve pool PK analysis were inputted for POPPK analysis. Based on -2LL (-2 log likely) and Akaiki information criterion (AIC), 2-compartment pharmacokinetic model with first order absorption best described as the base model for the pharmacokinetics of B1A10-2474. Multiplicative error model incorporated in the base model further significantly dropped the -2LL (dropped > 6.635) and AIC values in comparison to additive model. Stepwise covariate search by addition and followed by deletion of covariate, age, weight, brain weight and renal function for initial and terminal absolute oral clearance and volume distribution yielded no significant change in -2LL value. Results of bootstrap analysis are tabulated in Table 3 and goodness of fit plots for observed

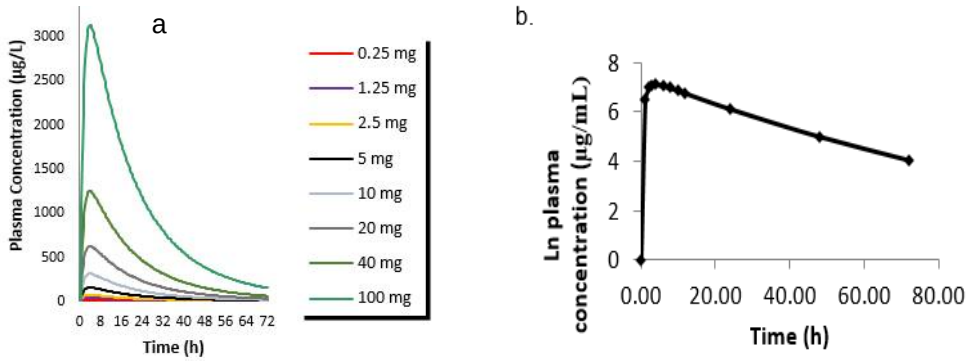

Figure 2: Simulated mean plasma concentration-time profiles for different single doses of B1A10-2474 in healthy males (N=10) (a). Simulated mean natural log plasma concentration (µg/mL) time profile for 40 mg single dose of B1A10-2474 in healthy male subjects (b)

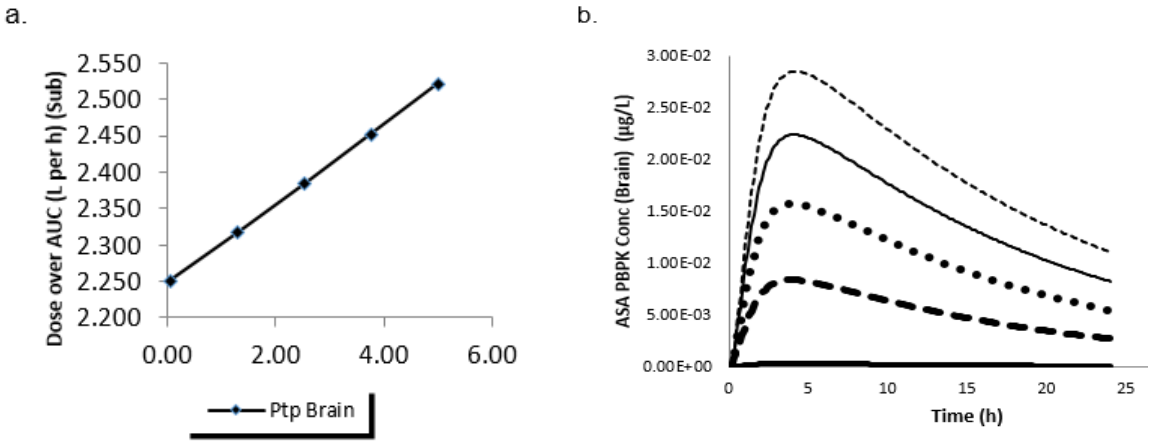

Figure 3: Brain tissue partition coefficient (Ppt) is linearly related to area under the curve (AUC) of the drug in plasma (a). Concentration of the drug achieved in brain is depended on the brain tissue partition coefficient (Ppt) (b)

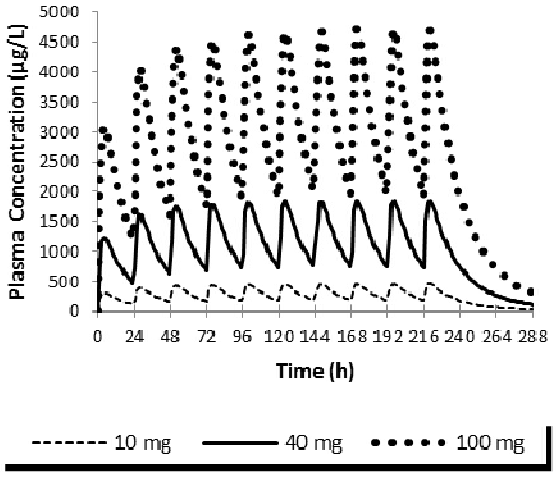

Figure 4: Simulated mean plasma concentration-time profiles of multiple doses of B1A10-2474 in healthy males (N = 10)

data to that of the individual and population predicted are pictorially presented in Figure 5. The final PK model for B1A10-2474 was 2-compartmental with first order absorption with multiplicative error.

DISCUSSION

B1A10-2474 showed linear pharmacokinetics for the range of the doses tested as the ratio of AUC to dose was constant for all the doses tested. However at the higher doses of 50 and 100 mg, half-time of the drug tend to increase.

Pharmacokinetics of B1A10-2474 exhibited 2 compartmental disposition. Exposure of B1A10-2474 in brain is high and is in a linear relation with the plasma concentration. Multiple dosing with B1A10-2474 for the doses tested didn't suggested possibility of drug accumulation. However ADMET data predicted that B1A10-2474 has potential to inhibit CYP3A4 mediated metabolism of midazolam and testosterone (data not shown), as B1A10-2474 itself is a CYP3A4 substrate there is a possibility that B1A10-2474 can inhibits its own metabolism which can be of importance in multiple dosing scenario. Because of the linearity of the concentration achieved in brain to the concentration in plasma, accumulation in blood due to auto inhibition of CYP3A4 may lead to the higher concentrations in brain.

Due to the lack of experimental data and non-availability of data of any kind with regards to B1A10-2474, this study presented simulated results based on the predicted physio-chemical properties from ADMET predictor. Because of

Table 3: Pharmacokinetics of single dose of B1A10-2474 (40 mg) obtained from bootstrap analysis

Parameter	Bootstrap results			
	Theta Estimate (mean)	CV%	2.5% CI	97.5 CI
tvKa	0.055	10.109	0.046	0.067
tvV	0.027	8.667	0.023	0.032
tvV2	7.92	40.59	6.06	19.63
tvCl	0.015	10.651	0.012	0.019
tvCl2	0.000154	71.3	0.000026	0.000395

tvka- typical value of co-efficient of absorption (Ka) ; tvV- typical value of volume of distribution of central compartment (V); tvV2- typical value of volume of distribution of peripheral compartment (V2); tvCl- typical value of initial clearance (Cl); tvCl2- typical value of terminal clearance (Cl2)

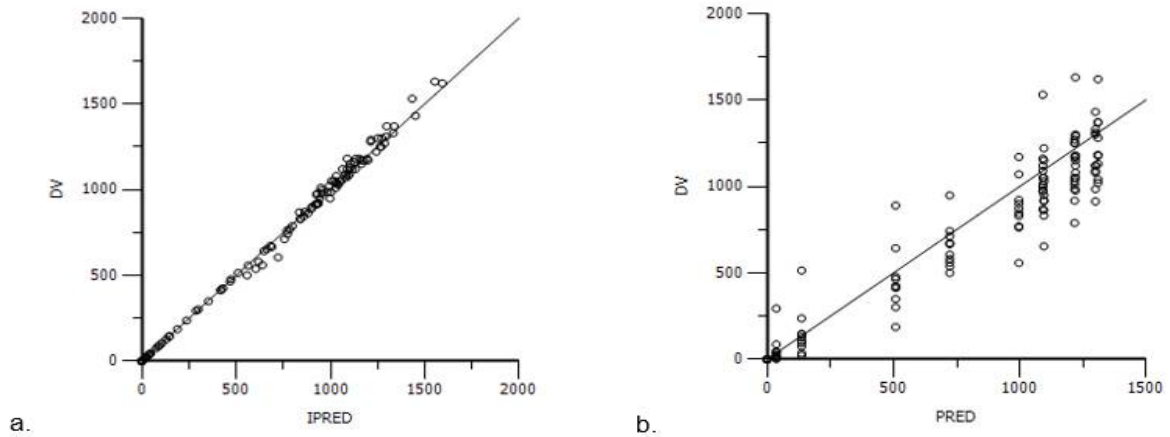

Figure 5: Dependent variable (concentration (Cobs)) versus individual predicted data (a) and population predicted data (b)

Table 4: B1A10-2474 caution data generated as a practical example for application of *in silico* methods to ascertain safety and a guide for further confirmatory safety requirements

Cautions flagged *from in silico* methods for B1A10-2474	**Possible data sought to elaborate on safety**
High brain permeability	• Extensive animal data to establish the safety of the drug in the brain tissue including histopathological investigations. • During clinical trials in human protocol inclusion of regular monitoring of brain safety.
Metabolized by CYP3A4	• Pharmacokinetic interactions data with the known inhibitors and inducers of CYP3A4 enzyme both *in vitro* and *in vivo*, if clinical relevance expected from *in vitro* data.
CYP3A4 Inhibition potential	• Chronic multiple dosing studies specially with high dose of the drug in animals. • During clinical trial protocol to reflect sequential dosing with the human subjects specially while progressing from lower doses to higher multiple dosing.
Delayed terminal elimination of the drug	• Concentration time profile of the drug concentration in vital tissues over the time specially with multiple dosing from *in vivo* animal experiments.

the non-availability of the experimental animal or human data, PBPK model utilized couldn't be validated against the real human data. As such results from this study would rather serve as a qualitative assessment of pharmacokinetics of B1A10-2474. However this study rendered some possible explanations for the fatality of the clinical trial with B1A10-2474, which can be taken as lessons learned from the trial to potentially use *in silico* approaches including PBPK modeling to flag a caution for early safety of drugs prior to use in humans.

France trial on B1A10-2474 was undertaken as per the standard protocol for clinical trials. Pharmaceutical company undertaking the trial sought the approval for conduct of the trial, had administered the single dose of the drug to about 90 healthy subject without any suspicion of serious adverse effects. However upon the multiple dosing regimen of 50 mg for about 3 days, 5 of the 6 subjects were reported to have serious injury to brain tissues which was not the case with lower multiple dosing [8]. Subsequently one of the subject was reported to be brain dead and 4 subjected to lifelong deformity. The Pharma Company was then accused of not being fair in disclosing the prior information of the trial, failure to register the trial with any trial registry, being secretive and failing to take appropriate measures after being aware of the unsafe nature of the drug under trial. Major criticism in conduct of the trial is failing to follow sequential dosing. B1A10-2474 seem to be a case of dose related drug accumulation in brain to the extent of tissue damage. I*n silico* methodology utilized in this study also implicated that B1A10-2474 showed the tendency of dose related accumulation as the drug have high brain permeability which is in linear relation to the dose, a slow terminal clearance and speculation of auto-inhibition of its metabolism.

TeGenero trial had similar consequences which nearly killed 6 healthy subjects due to involvement of immune system (6) that lead to multiple organ failure [7]. In view of the above recent disasters in phase I clinical trials, despite the advancements in drug development process noticed in this decade, caution should be taken to avoid such events in future.

Regulatory bodies should take the extra precautionary steps in approving and strictly advising close monitoring of patients and observing adequate dose spacing between subjects in clinical trials. Moreover, *in silico* processes can be performed independently by the regulatory agencies merely with computing facilities. *In silico* cannot replace the experimental data however, it can be a useful guide to regulatory agencies to demand for data which addresses the safety or efficacy of the drug. For example in this study, use of *in silico* approaches cautioned B1A10-2474 as a drug of high brain permeability, a substrate of CYP3A4 enzyme and potential to inhibit CYP3A4. Based on these cautions regulatory agencies can demand for specific data to ascertain the safety of the drug. Table 4, elaborates the cautions flagged by the *in silico* approaches utilized in this study for the safety of B1A10-2474.

CONCLUSION

This simulation study puts B1A10-2474 as a high alert drug based on the fact that penetration of the drug in brain is high and linear with the dose administered, drug with delayed terminal clearance and drug that have potential to

accumulate in human body over time with multiple dosing possibly due to inhibition of CYP3A4 enzyme. *In silico* approaches used this study generated caution which were noticed in the fetal clinical trial with B1A10-2474. Lessons learned from the applications of the *in silico* approaches to understand the possible reasons for safety failure of B1A10-2474 can be extended to other drugs under development.

DECLARATIONS

Acknowledgement

Author acknowledges Dr Atul Rathorde's contribution for generating ADMET predictor data for B1A10-2474. Author is also thankful for proof reading services rendered by ScribeFast, scribe-fast.com.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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