Combining *In Vitro-In Vivo* Extrapolation (IVIVE) and Physiologically-Based Pharmacokinetics (PBPK) with Drug Related Risk Assessment: Putting Pieces Together for A Priori Assessment of the Likelihood of Cardiotoxicity

Sebastian Polak* (1,2), Masoud Jamei (1), Amin Rostami-Hodjegan (1,3)

spolak@simcyp.com

(1) Simcyp Ltd., Blades Enterprise Centre, Sheffield, UK; (2) Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland; (3) School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Road, Manchester, UK;

Introduction Cardiotoxicity assessment is a compulsory element of the drug development process required by the drug agencies including FDA and EMA [1,2]. The risk of fatal ventricular arrhythmia triggered by drugs is the rationale for such requirements. Over the last two decades prolongation of QT interval has been responsible for the withdrawal of several drugs from the market and the extensive black box labeling restrictions. Although there are a number of mechanisms by which drugs may lead to QT prolongation, inhibition of the potassium channels encoded by the hERG gene has been a cause in many cases and interaction with hERG channel has become a major pharmacological safety concern. The potential pharmacological and toxicological effects – except the idiosyncratic reactions – are dose dependent. IVIVE incorporated into PBPK provides the opportunity of simulating variability of ADME in large populations, 'prior' to conduct of clinical trials. Combination of this approach with any information on concentration-effect relationship may assist with identifying the likelihood of observing pharmacological or toxicological effects, an essential step in study design.

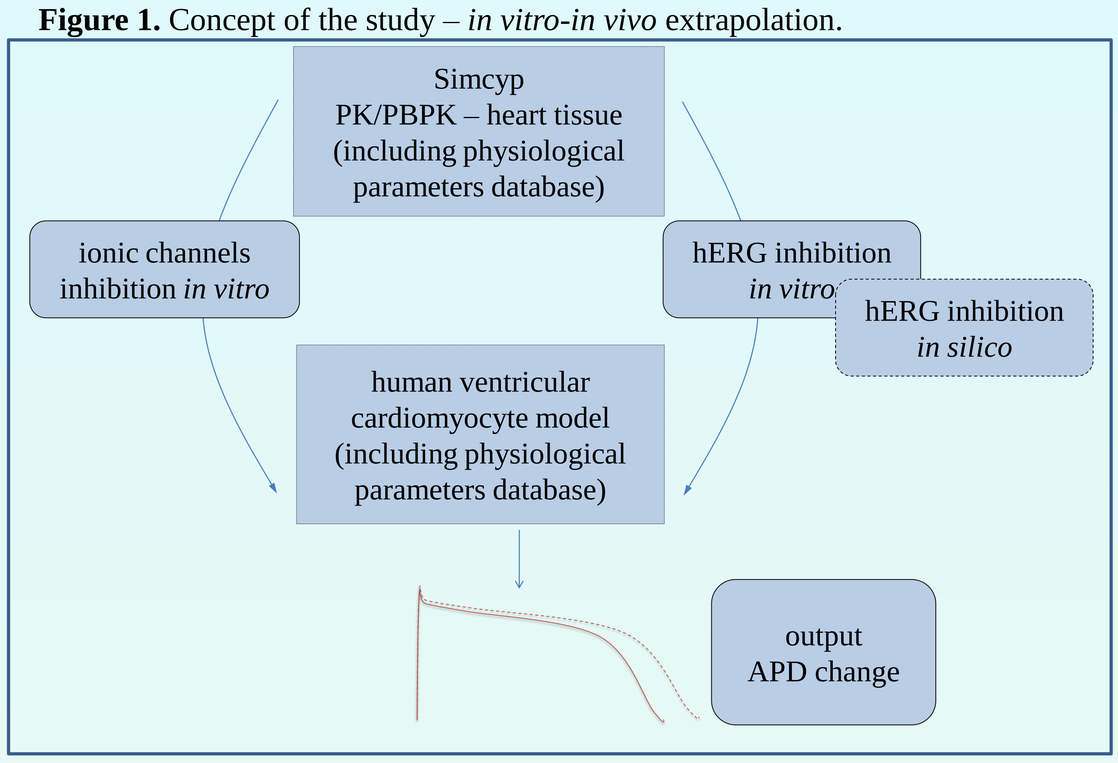
Objectives. The main objective of the current project was to assess potential cardiotoxic risk, including inter-individual variability, by combining the *in silico* mechanistic cardiomyocyte model and PBPK models. Clozapine as a non-cardiology drug has been associated with potential harmful cardiac side effects [9]. This drug was used as an example.

Methods Simcyp V10.1 IVIVE/PK platform was used for the prediction of clozapine concentrations in plasma and heart tissue after oral dosing. Simulation settings were as follows:

- *single 25 mg oral dose*
- *first order absorption (ka = 2.22 [h-1])*
- *full PBPK disposition distribution - heart tissue-to-plasma partition coefficient was calculated to be 6.16 [3]*
- *North European Caucasian - 6 individuals as presented in the tables below, to assess inter-individual variability in PK*
- *plasma and heart tissue concentrations as the operational concentrations for the pharmacodynamic effects.*

To account for inter-individual variability of toxicity, population was further described by combination of age dependent physiological covariates, namely cardiomyocyte volume, cardiomyocyte area, sarcoplasmic reticulum volume, cell electric capacitance [4]. Partial output was described as drug concentration dependent ionic channels inhibition and was transferred further to the human ventricular cardiomyocyte model of the human ventricular epicardium cell [5] implemented in CompTox system [6]. Simulation time and the maximum time step were set to 400 ms and 0.1 ms respectively. Final endpoint was defined as the action potential duration and its

Figure 1. Concept of the study – *in vitro-in vivo* extrapolation.



Results Maximum plasma and heart tissue concentrations, different for every individual, were chosen as the operational concentration. Information regarding the clozapine influence on the ionic channels was derived from the literature and is presented below (Table 1) [7,8].

Table 1. Virtual individuals and drug specific information.

Individual				Cardiomyocyte			Drug			
Index	Age (Years)	Sim Heart Cmax (µM)	Sim Plasma Cmax (µM)	Volume (μm^3)	Area (μm^2)	Capacitance (pF)	I_{Kr} inh [%] Heart/Plasma	I_{k1} inh [%] Heart/Plasma	I_{to} inh [%] Heart/Plasma	I_{Na} inh [%] Heart/Plasma
1	21	1.69	0.29	4567	1702	33.92	78.4 / 50.2	0.0 / 0.0	0.7 / 0.1	14.1 / 4.8
2	33	1.19	0.21	8113	2356	46.93	73.8 / 44.3	0.0 / 0.0	0.4 / 0.0	11.5 / 3.9
3	47	2.32	0.38	12110	3456	68.85	82.1 / 55.1	0.1 / 0.0	1.1 / 0.1	17.0 / 5.7
4	43	2.72	0.46	19822	4488	89.41	83.7 / 58.5	0.1 / 0.0	1.3 / 0.1	18.5 / 6.4
5	25	2.69	0.45	5399	1604	31.95	83.6 / 58.1	0.1 / 0.0	1.3 / 0.1	18.4 / 6.3
6	36	2.12	0.36	7842	2048	40.79	81.1 / 54.1	0.1 / 0.0	1.0 / 0.1	16.1 / 5.5

It was assumed that inhibition observed *in vitro* is equal to simulated *in vivo*. Channels inhibition for maximum concentration for various patients was calculated after fitting experimental data to the Hill function.

Table 2. Virtual clinical trial results.

Individual		APD_{90} [ms]							
		Heart tissue				Plasma			
Index	Age (Years)	Without Clozapine	With Clozapine	Difference	Change compared to native [%]	Without Clozapine	With Clozapine	Difference	Change compared to native [%]
1	21	160	170	10	6.3	160	166	6	3.8
2	33	161	171	10	6.2	161	167	6	3.7
3	47	162	173	11	6.8	162	169	7	4.3
4	43	164	175	11	6.7	164	171	7	4.3
5	25	160	172	12	7.5	160	168	8	5.0
6	36	161	172	11	6.8	161	168	7	4.3
mean	**34.17**	**161.33**	**172.17**	**10.83**	**6.7**	**161.33**	**168.17**	**6.83**	**4.2**
SD	**10.05**	**1.51**	**1.72**	**0.75**	**0.5**	**1.51**	**1.72**	**0.75**	**0.5**

Figure 2. Simulated drug concentration change in time for six individuals.

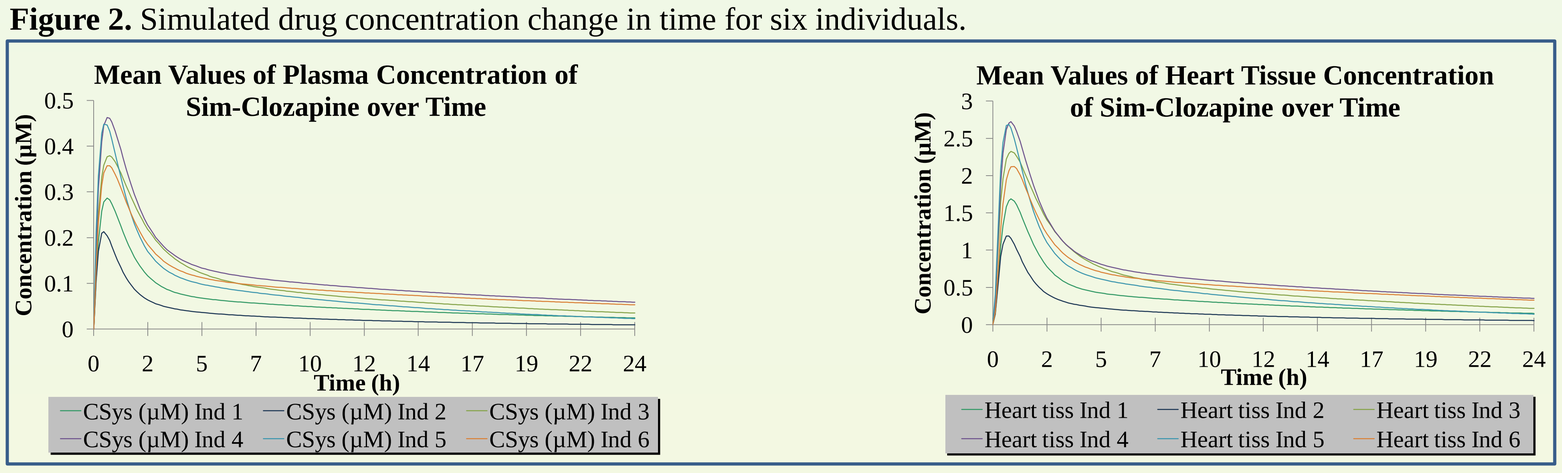
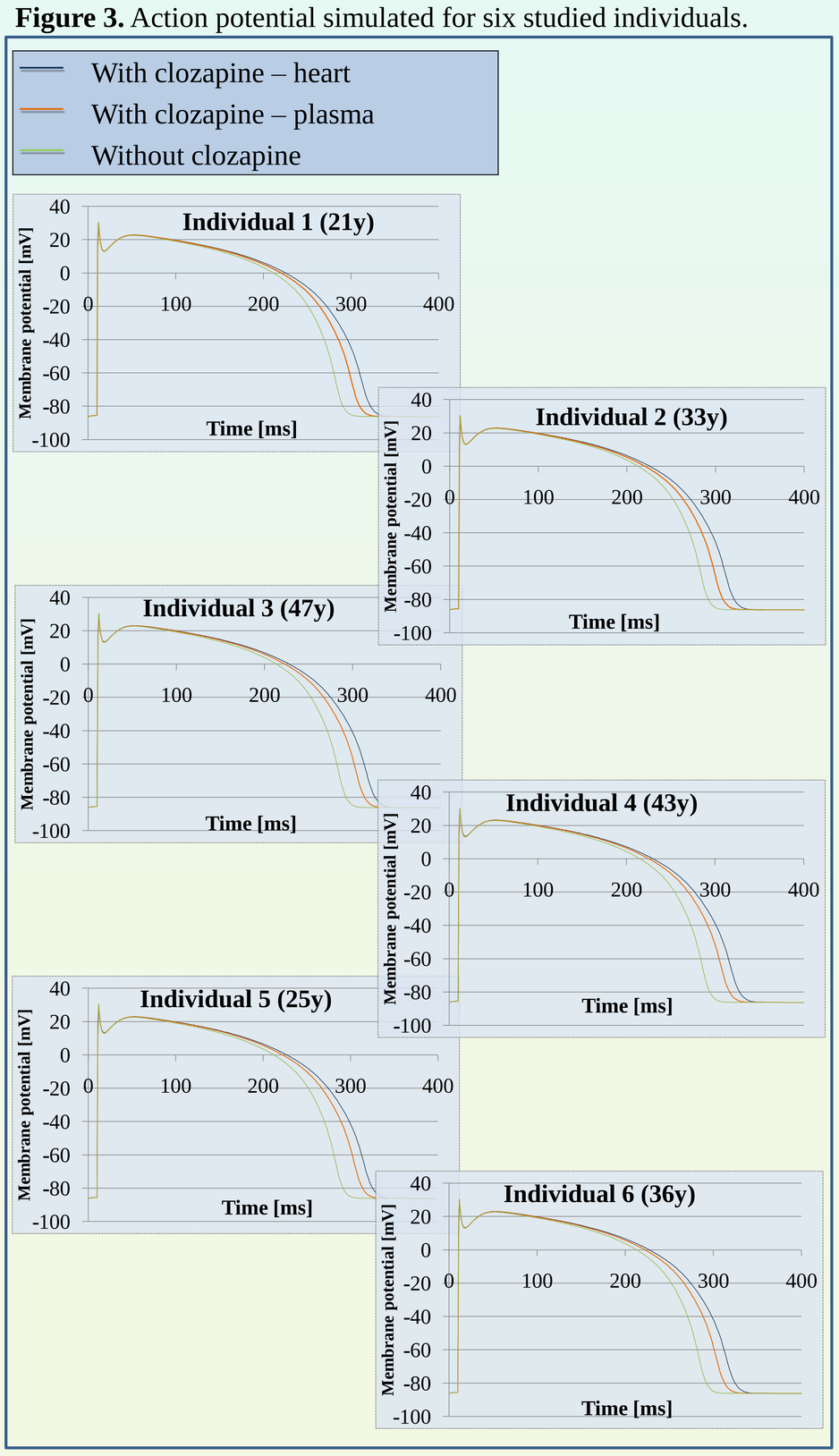


Figure 3. Action potential simulated for six studied individuals.



Discussion Results presented above need to be interpreted further to gain some conclusions which could be relevant for the drug development specialists. One of the few possible ways is to calculate and analyze pseudo-ECG rather than AP only. Obtained results were compared with clinical observations for clozapine. Predicted increase in APD_{90} (~7%) doesn't suggest substantial increase in QT length although some influence might be observed. As it was recently published by De Ponti there are some clinical evidence supporting thesis about clozapine influence on QT but the prolongation potential is low [9]. Among other factors influencing drugs QT prolongation potential genetic element plays a significant role. There is an ongoing project which aims at incorporating genetic variability to the virtual population and assessing quantitatively its impact.

Project partly financed by the Polish National Center for Research and Development
LIDER project number LIDER/02/187/L-1/09

References

[1] http://www.fda.gov/cder/guidance/5533dft.pdf
[2] http://www.emea.eu.int/pdfs/human/ich/000204en.pdf
[3] T. Rodgers, M. Rowland, *J Pharm Sci* (2006) 95(6): 1238-1257.
[4] http://www.tox-portal.net
[5] H.W.J. Ten Tusscher et al., AJPhys Heart Circ Phys (2004) 286(4): H1573-H1589.
[6] http://www.tox-comp.net
[7] W.J. Crumb Jr. et al., *Pharm Research* (2006) 23(6): 1133-1143.
[8] S.-Y. Lee et al., *Br J Pharmacology* (2006) 148(4): 499-509.
[9] F.D. Ponti et al., *Drug Safety* (2002) 25(4): 263-286.