Optimal cancer chronotherapeutic schedules of Seliciclib revealed by a systems biology approach

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SIXTH FRAMEWORK PROGRAMME

1 - Circadian regulation and Chronotherapy

Chronotherapy consists of coordinating the timing of a medical treatment with a patient's biological rhythms in order to optimise a drug's beneficial effects and reduce the undesired ones. The TEMPO project [1], part of the EU 6th Framework Program, specifically concerns the design of optimal chronotherapeutic schedules in an attempt to improve both the efficacy and tolerability of the cancer therapeutics Seliciclib and Irinotecan.

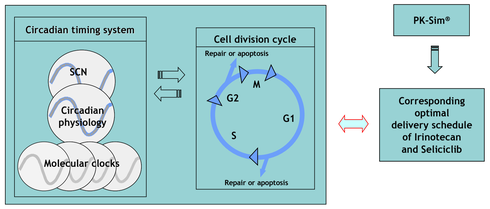

Fig. 1 Therapeutic implications of the interactions between the circadian timing system, the cell division cycle and the pharmacology determinants. SCN: suprachiasmatic nuclei, the main circadian pacemaker in the hypothalamus.

In order to study the optimal chronotherapeutic delivery patterns of the anti-cancer drug Seliciclib in the mouse, we combined several technologies: our proprietary cell cycle model, which was coupled to a circadian clock model; a simulated PK profile of Seliciclib in mouse; and a PK/PD combined model.

2 - Core Cell Cycle Model

The **eukaryotic cell cycle** is usually divided into four phases (→ G1 → S → G2 → M →): the S-phase (synthesis -- DNA replication), the M-phase (mitosis, cytokinesis) and two gaps phases G1 and G2 (Fig. 2 & 3).

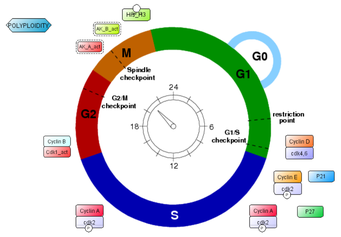

Fig. 2 Schematic representation of the cell cycle and its main players.

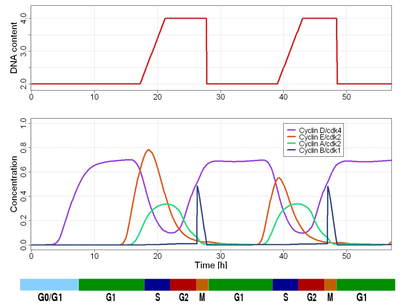

Fig. 3 Two wild-type mammalian cell cycles starting from G0 arrest: DNA replication (top) and time courses of cyclins E, D, A & B in complex with cdks (bottom) are shown. The simulations were performed in Jarnac – a metabolic simulation package.

3 - SystemCell® cell population simulator

SystemCell® is a **cell population simulator**. Each cell is an instance of an autonomous cell cycle model combined with a discrete event-based state machine, which together determine the cell's fate including proliferation, polyploidy, or apoptosis (Fig. 4). Several implementations of SystemCell® have been developed, one of them on a High Performance Computer "Blue C" available at Institute of Life Science, University of Swansea [2]. Code parallelism confers an increase in performance of SystemCell® by a factor ~30 compared with single-processor implementation.

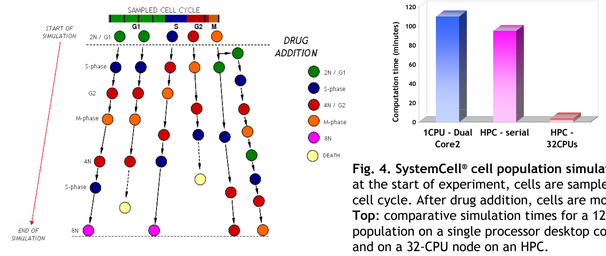

Fig. 4. SystemCell® cell population simulator. Left: at the start of experiment, cells are sampled over a cell cycle. After drug addition, cells are monitored. **Top**: comparative simulation times for a 128 cell population on a single processor desktop computer and on a 32-CPU node on an HPC.

SystemCell® allows the monitoring of any kind of variable in the population, reproducing typical experiments such as FACS (monitoring the overall DNA content), the phosphorylation state of biomarkers or the mitotic index.
SystemCell® is also able to simulate the "injection" of a drug concentration-time profile into the cell population, providing a **fully integrated PK/PD simulation framework** (Fig. 5).

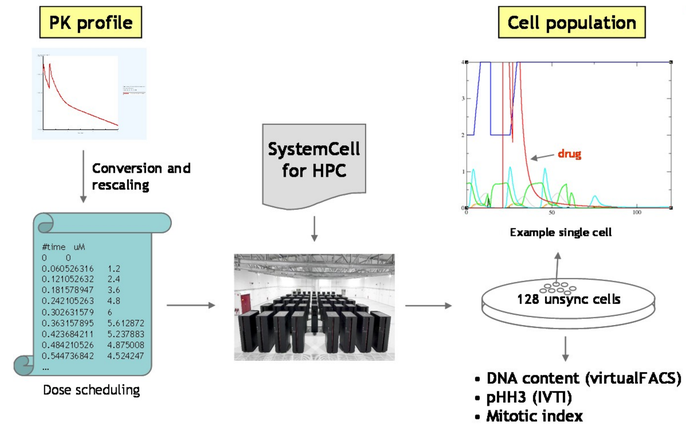

Fig. 5. PK/PD simulations using the SystemCell® cell population simulator. The PK profile of a drug can be injected into the PD simulation.

4 - Circadian clock model and coupling

Physiomics' proprietary cell cycle model [3] was **coupled to a circadian clock model** through the G2/M transition point by regulating the balance between Wee1 and Cdc25c activities (based on [4-5]). This allowed the synchronisation of the cell cycle model with a 24-hour circadian rhythm and showed that Physiomics' model was able to use the oscillations of the circadian regulator as its internal clock (Fig 6). Cyclic Bmal1 and Per expression profiles can be related to the 12 hour long dark and light phases (shown as black and white rectangles in Fig. 6), in turn relating this to the different cell cycle phases.

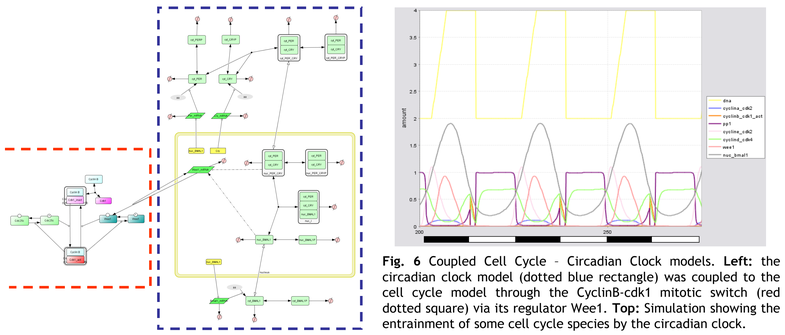

Fig. 6 Coupled Cell Cycle – Circadian Clock models. **Left:** the circadian clock model (dotted blue rectangle) was coupled to the cell cycle model through the CyclinB-cdk1 mitotic switch (red dotted square) via its regulator Wee1. **Top:** Simulation showing the entrainment of some cell cycle species by the circadian clock.

5 - Seliciclib PK simulations

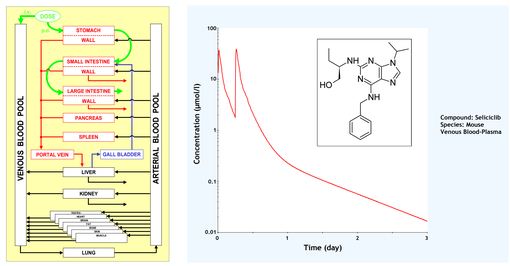

Fig. 7 Left: Whole-body model used in PK-Sim® for the simulation of drug concentration-time profiles **Right:** Simulated plasma exposure for a double administration of Seliciclib in mouse. The profile corresponds to two 50mg/kg dose injection separated by 6 hours. Insert shows chemical structure of Seliciclib (R-roscovitine, CYC202) [9].

PK modelling of Seliciclib was performed using PK-Sim® [6], a whole body simulation software that is pre-calibrated for mouse, rat, dog and human, allowing detailed organ levels of a drug to be predicted (Fig. 7). Using initial physicochemical data and properties of Seliciclib that were gathered from publications, plasma exposure was calculated for single and multiple dose drug administration in mouse. There was good agreement between the simulated profile and published data (not shown).

6 - Chronotherapeutic scheduling using PK / PD simulations

Combined PK/PD simulations were performed to evaluate the impact of a multi-dose injection of Seliciclib in a single cell or cell population after administration at different times.

At the level of **a single cell**, the effects of a single dose of Seliciclib were evaluated after administration at different stages of the cell cycle (Fig. 8). Seliciclib effect is modelled in our coupled circadian clock - cell cycle model as **a competitive ATP inhibitor of CDK activity**, using inhibition terms (IC50 values) which have been incorporated in the kinetic rates of the model equations, as described in [3]. Using this technique, optimal periods of administration of the drug were established. When administered at early G1 cell cycle progression was barely affected, whereas administration during the S phase or at G2/M transition resulted in complete cell cycle arrest.

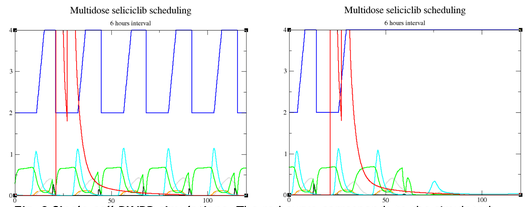

Fig. 8 Single cell PK/PD simulations. The red curve corresponds to the simulated plasma PK profile of Seliciclib as calculated in Fig. 7. **Left:** drug is injected at the start of the cell cycle, leading to barely no effect on the cell cycle progression. **Right:** drug is injected in late G1, leading to rapid cell-cycle arrest.

At the level of **a cell population** (Fig. 9), two administration times were used: ZT3 and ZT19 (3 hours and 19 hours after start of light phase, respectively). At the start of simulation, each cell is an individual instance of the model and the whole population can represent different levels of synchronisation. PK/PD simulations were performed to evaluate the impact of a single and multi-dose injection of Seliciclib on synchronous and asynchronous cell populations, as they can reflect the difference between healthy tissue (where cells are entrained by the circadian clock) and tumour tissues (where cells divide asynchronously) [7-8].
In a synchronised tissue, administration at ZT3 has no effect as all cells exposed to the drug are at the beginning of the G1 phase. At ZT19 all cells are arrested as they are in late G1 phase. Conversely, in an asynchronous tissue, a proportion of cells are sensitive to the drug at both ZT3 and ZT19. These results are in line with experimental measurements [7].

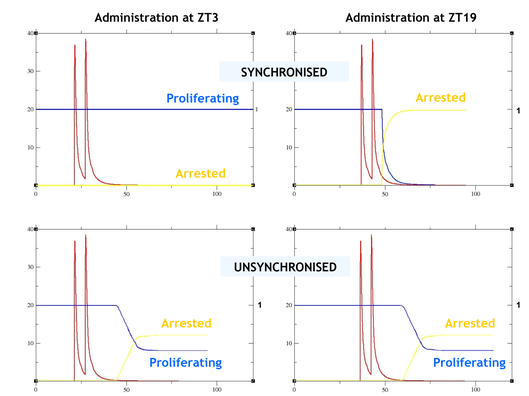

Fig. 9 Multiple cell PK/PD simulations. Drug (red curve) is injected at different ZT times in populations of synchronised (top row figures) or unsynchronised (bottom row figures) population of cells. The blue curve corresponds to the proportion of cells that continue to proliferate, whereas the yellow curve correspond to arrested cells. ZT time is determined according to the levels of Per/Cry and Bmal proteins in the population.

Choosing different drug administration times is clearly critical to optimise the therapeutic index of the drug, ensuring the maximum effect on tumour tissue but a minimum effect on healthy, synchronised tissue. The simulations here pave the way for the design of optimal chronotherapeutic administration schedules for Seliciclib, which will allow the selective targeting of tumour cells and sparing of healthy cells, maximising efficacy and reducing toxicity.

7 - Conclusions

The general objective of TEMPO is to design *in silico* and *in vivo* mouse models reflecting different dynamic classes that allow the prediction of distinct optimal chronotherapeutic delivery schedules. Physiomics' technology allows the simulation of the reciprocal interactions between the circadian clock and the cell division cycle, and their combined interaction with PK processes. This permits the design of optimal chronotherapeutic delivery schedules of Seliciclib. Although the scope of the TEMPO project is limited to the creation of *in silico* models of the mouse, it is envisaged that the chronotherapy models generated in TEMPO will later be applied to humans. The ultimate goal is the personalisation and specific tailoring of cancer chemotherapy for different patient classes.

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