Systems biology analysis of CYC116, a novel aurora kinase inhibitor

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Introduction

Aurora kinases are known to play a role in mitotic entry, progression and exit. These kinases represent attractive targets for anticancer drug development because they regulate both genomic integrity and cell cycle progression in cancer[1].

Cyclacel and Physiomics entered a collaboration to model the action of Aurora kinase (AK) inhibitors, focusing on describing the effects of the phase I dual Aurora A and B inhibitor CYC116 and other Cyclacel inhibitors on cell cycle progression. Using both published data and data supplied by Cyclacel for its proprietary compounds, a dynamic and accurate cell cycle simulation was extended to include the key actions of AKs, in order to simulate the various aspects of their inhibition. Whereas AK-A regulates centrosome cohesion and separation, AK-B is responsible for maintaining active the spindle checkpoint (SCP) and triggering cytokinesis.

The result of this collaboration is a realistic and precise model of the cell cycle progression, particularly useful for the study of the effects of cancer drugs.

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. 1 & 2).

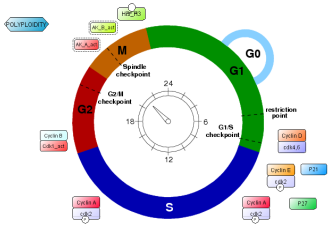

Fig. 1: Schematic representation of the cell cycle and its main players.

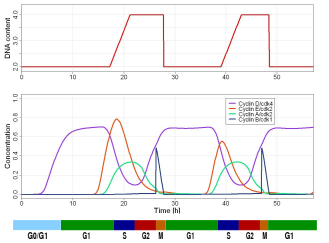

Fig. 2: Two wild-type mammalian cell cycles starting from G0 arrest: DNA replication (top) and time courses of cyclins E, D, A & B in complex with cdks (bottom) are shown. The simulations were perfor-med in Jarnac - a metabolic simulation package.

Histone H3 Phosphorylation as AK B Functional Biomarker

Aurora B is known to phosphorylate Histone H3 on Serine 10. Using **histone H3 as a biomarker for polyploidy** (Fig. 3a), we calibrated the cell cycle model so that the level of phosphorylation of histone H3, controlled by AK B, triggers the polyploid state in A549 cells [2]. To calibrate the model, we used CYC116 data and data published for VX-680. Using the parameter set obtained for CYC116, the model was able to predict without any fitting the experimental histone H3 phosphorylation IC50 for a series of Cyclacel compounds from the measured Ki for AK A and AK B (Fig. 3b). These results confirm that Histone H3 is a good biomarker of AK B activity and can be used as a predictor of polyploid status.

Fig.3. a Aurora Kinase A & B module, connections to Histone H3, drug injection and polyploidy (the diagram was created with CellDesigner® a structured diagram editor for gene-regulatory and biochemical networks).

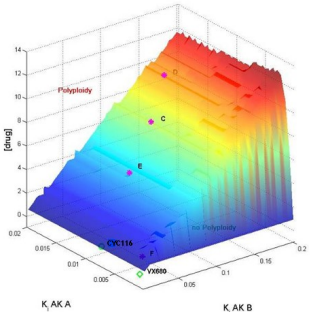

Fig. 3. b Simulation of Histone H3 phosphorylation threshold for polyploidy vs AK A IC50, AK B IC50 and drug concentration for Cyclacel AK inhibitors

SystemCell® cell population simulator

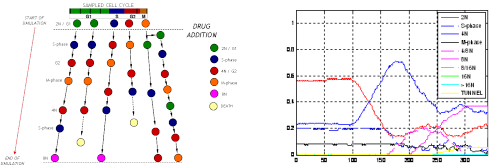

Fig. 4. SystemCell cell population simulator. Left: at the start of experiment, cells are sampled over a cell cyle. After drug addition, cells are monitored. **Right**: Simulation results, showing the DNA content of the population over time.

SystemCell is a **cell population simulator**. Each cell is an instance of an autonomous cell cycle, combined to a discrete event-based state machine, which determines its fate from proliferation, polyploidy state to the multiple stages of apoptosis in a stochastic way (Fig. 4). Another layer of stochasticity was added, taking into account differences of the different cells in sensitivity to the drug. By monitoring the DNA content in the population, the simulation generates an output equivalent to a FACS experiment.

Simulating CYC116 effect

SystemCell technology was used to analyse the **effects of the inhibition of Aurora kinases A and B on a population of A549 cancer cells**. Using Cyclacel data, SystemCell was calibrated for various Aurora kinase inhibitor compounds. Cell cycle models used in SystemCell focused primarily on the effect of the inhibition of Aurora kinase B. We demonstrated we were able to reproduce the effects of various inhibitors of AKB and in particular for CYC116 (Fig. 5). Moreover, we have been able to predict the effect of other compounds on A549 cells using a wide concentration range.

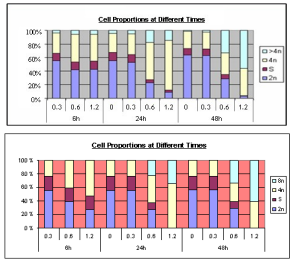

Fig. 5. Effect of CYC116 on cell cycle distribtion of A549 cells. Top: Experimental values. **Bottom**: Simulation results.

Auto synergistic/antagonistic effect of the drug

Studies to investigate **the effects of combining individual drugs** are common, but not often have the potential target-based drug-drug interactions to be taken into consideration. Similar to microtubule agents, the inhibition of Aurora A is expected to induce a spindle checkpoint-induced mitotic delay which will then trigger a cell death response. In contrast, the inhibition of Aurora B is expected to disable the spindle checkpoint and result in a rapid exit from mitosis and a failure of cell division. Dual Aurora A/B inhibitors could therefore be expected to act differently depending on whether the A inhibitory effect is dominant over B or not. In the example presented in Fig. 6a the AK A component of the drug is prominent up to 3 µM. In this concentration range, the SCP is activated leading to a slower degradation of cyclin B and a delayed mitotic exit. Above 3 µM (see Fig 6b), the AK B component is prominent leading to an override of the SCP and an accelerated exit from mitosis. The incidence of these concentration-dependent mode of actions is particularly important when considering the different cell death response they will induce (see Scaerou *et al.*, EORTC-NCI-AACR 2006).

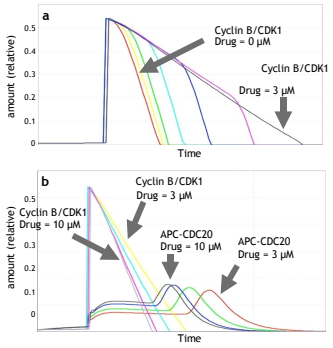

Fig. 6. a Cyclin B-CDK1 time-course for AK B inhibitor concentration less than 3 µM. **b** Cyclin B-CDK1 and APC-CDC20 time-course for AK B inhibitor concentration greater than 3 µM.

VirtualFACS simulation of mitotic slippage

SystemCell technology was also used to simulate **nocodazole override experiments** in modelled cancer culture of HeLa cells (Fig 7). The effect of combining nocodazole, which leads to microtubule depolymerisation, and AK inhibitors was thoroughly studied and modelled. In order to accurately model the effect of the combination of the drugs, the mitotic slippage effect (triggering exit from mitosis after a fixed period) was added to our cell cycle model. The model was able to reproduce the results obtain for two dual AK A/B inhibitors. CYC116 triggers a spindle checkpoint override (faster mitotic slippage) due to the AK B inhibition for this range of inhibitors concentration, whereas compound B, a more selective Aurora A inhibitor, shows no effects on mitotic slippage.

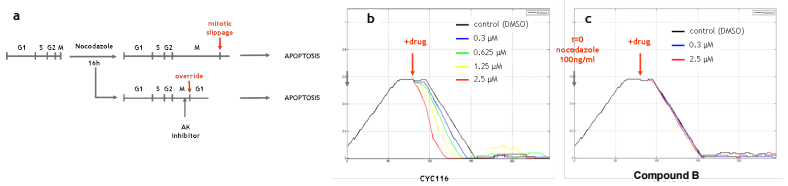

Fig. 7. a Mechanism of mitotic slippage after addition of nocodazole. Adding an AK B inhibitor will lead to an early exit of mitosis (override). **b & c** VirtualFACS simulation of mitotic index for a HeLa cell population after addition of nocodazole (100ng/ml) at t=0h followed by addition of an AK inhibitor at t=16h.

Conclusions

We present several results illustrating the responses of cancer cells to treatment with CYC116, a potent Aurora Kinase inhibitor. In our model, the Ki values and Aurora A and B of the candidate inhibotors can be used to predict candidate compounds MoA on cell cycle and mitotic progression, polyploidisation and other drug effects. The AurA/B inhibitory profile of the candidate inhibitors can also be useful to predict the concentration dependent mechanism of action of the compounds.

References:

[1] Keen, N. and Taylor, S., *Aurora Kinase inhibitors as anticancer agents*, (2004) Nature Reviews, 4, 927-936.

[2] Gizatulllin, F., et al., *VX-680, a small molecule inhibitor of Aurora Kinases induces endoreplication and apoptosis preferentially in p53 and p21 deficient cells*, Poster LB-238 AACR Meeting 2004.

[3] Harrington, E. A., et al., *VX-680, a potent and selective small-molecule inhibitor of the Aurora Kinases, suppresses tumor growth in vivo* (2004) Nat Med, 10, 262-7.