

Model-Based Analysis of RO7589831 Mechanisms of Action in a MSI Cell Line



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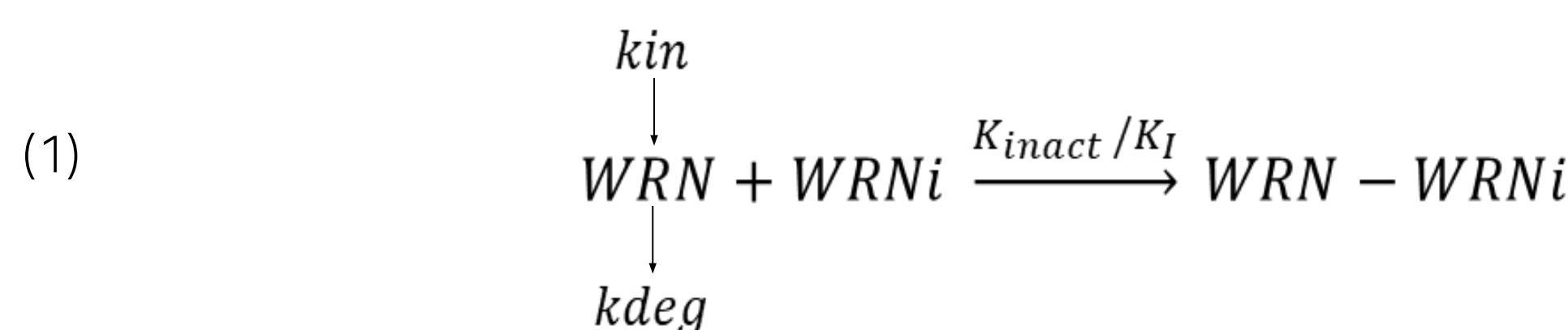
Background

- Werner (WRN) helicase is a synthetic lethal target in microsatellite instable (MSI) cancer cells, inducing cell cycle arrest and apoptosis when depleted [1,2].
- RO7589831 is a novel covalent WRN helicase inhibitor (WRNi) that selectively triggers G2/M phase arrest and apoptosis in MSI cells[2].
- An *in silico* model-based approach is used to understand the kinetics of WRN helicase inhibition and its effects on cell cycle and cell growth inhibition, enhancing knowledge of RO7589831's mode of action.

Methods: Modeling framework

WRN Target Engagement (TE) Model

To simulate the inactivation of the WRN protein by RO7589831, a covalent inhibition model was implemented [3], following reaction (1).



Where WRN is the amount of free WRN Helicase, k_{deg} represents the degradation rate of the WRN helicase, k_{in} is the synthesis rate of the protein, K_I is the binding affinity and the K_{inact} is the maximum potential rate of inactivation. This model was calibrated with *in vitro* binding data of RO7589831 to WRN helicase in HCT116 cell lines.

Mechanistic Cell Cycle Model

- A mechanistic model of the cell cycle was developed [4] (Figure 1), comprising four compartments and representing populations of cells through different phases of the cell cycle: G1, S, G2/M, and a G2 damage compartment (G2D) to simulate cell accumulation in the G2 phase as observed in preclinical data.
- WRN Inhibitor mechanism: The amount of free WRN helicase levels modulate the transition from S to G2 phase with damage.
- Parameters: Include transition rates (k_1 , k_2 , k_3), death rate in G2 damage (k_5), repair rate (k_4), and probability of damage during G2 (q).
- The cell cycle model was calibrated using HCT116 derived *in vitro* cell viability and cell cycle data, at different dosing and washout times.

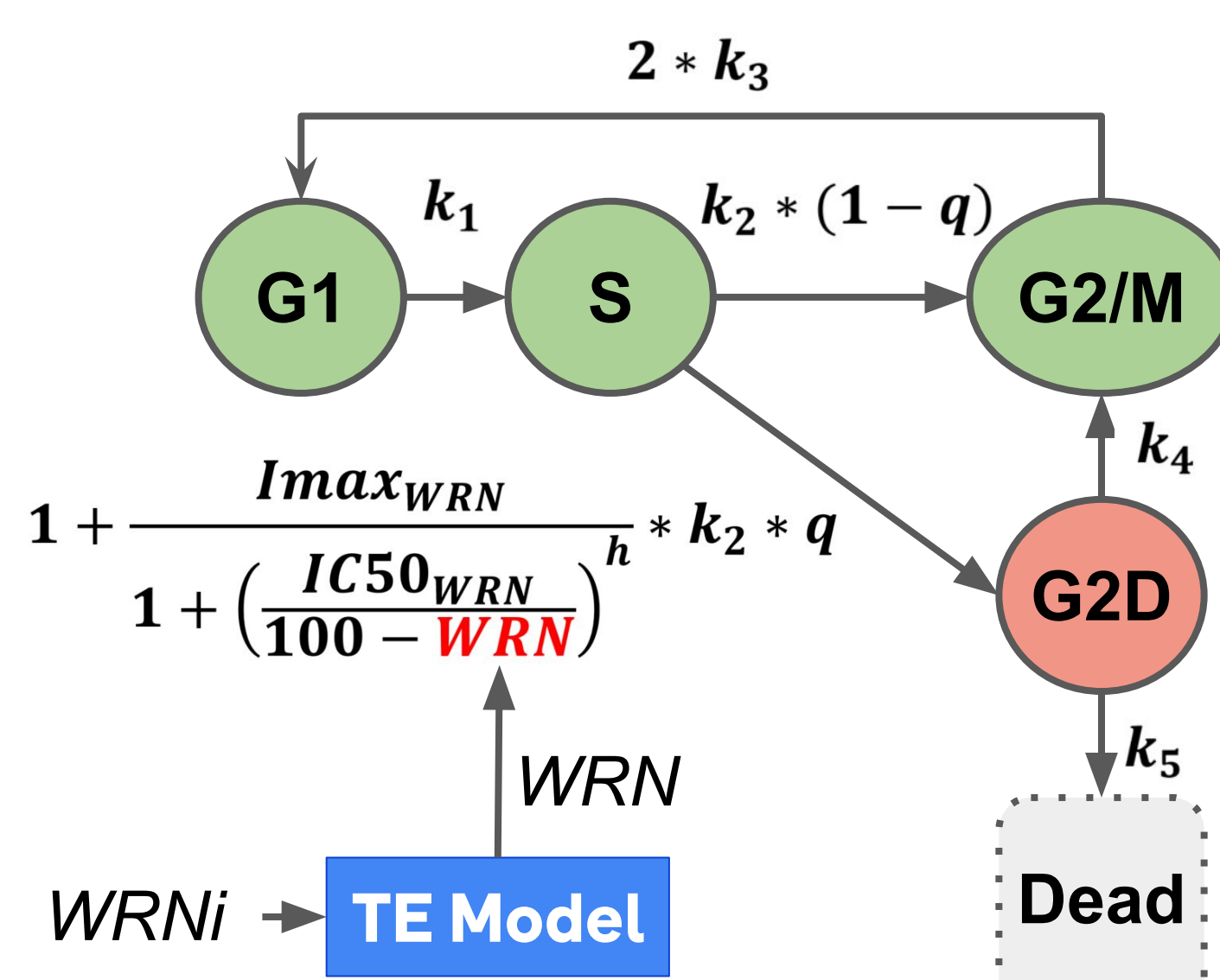


Figure 1. Mechanistic cell cycle model integrated with the target engagement model

Methods: *In vitro* experimental data

Three types of data were used for model calibration:

- Target Engagement Kinetics:** Mass spectrometry was used to estimate target engagement by measuring the loss of abundance of the free peptide in WRN engaged by RO7589831 using HCT116 cells, for concentrations between 1-1000nM, at time points between 5 and 240 minutes (Figure 2). Additionally, a washout experiment was performed to determine the kinetics of WRN recovery after drug treatment, incubating HCT116 cells with RO7589831 at 1-1000 nM for 2 hours, washed, and re-incubated in drug-free medium. Target engagement was measured 0 to 48 hours after washout (Figure 3).
- Cell Cycle Analysis:** HCT116 cells were treated with RO7589831 at different concentrations, and flow cytometry was used to estimate cell population across G1, S, and G2 phases based on DNA content of cells (Figure 4).
- Cell viability data:** HCT116 cells were treated with RO7589831 at concentrations ranging between 1 to 10000 nM, followed by washouts performed from 2 to 48 hours after compound treatment. Cell viability was determined 6 days after the start of the treatment (Figure 5).

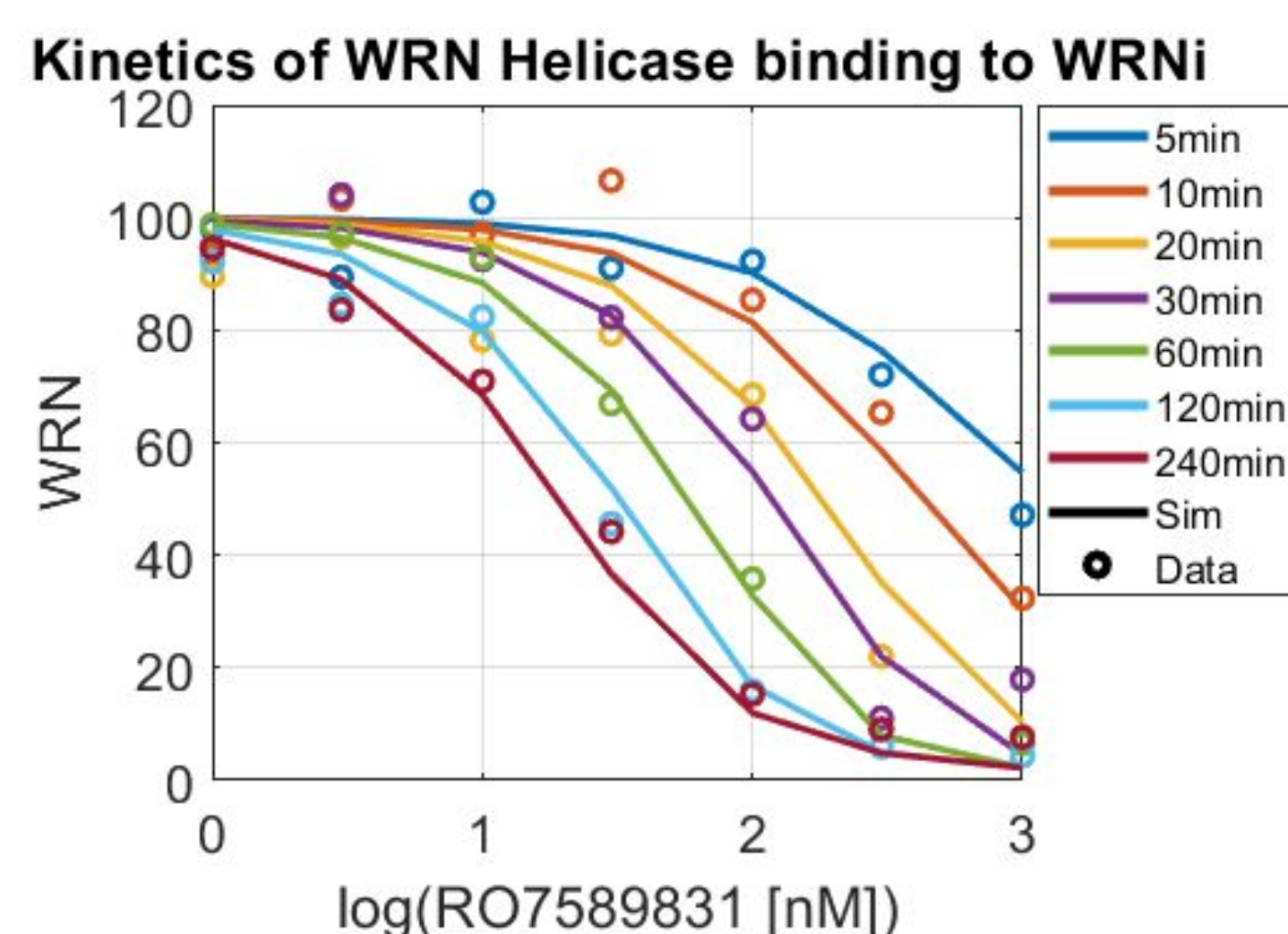


Figure 2. Fitting of simulated TE and experimental data of WRN helicase kinetics for different concentrations and at different timepoints.

Results

- Figures 2 and 3 illustrate the ability of the WRN TE model to replicate WRN kinetics in the presence of different concentrations of RO7589831, achieving a relative root mean square error (rRMSE) of 9.05% between simulated and experimental data. The TE model reproduces the rapid dynamics of WRN helicase inhibition in Figure 2. In Figure 3, the TE model captures the recovery of WRN helicase levels following a removal of RO7589831.

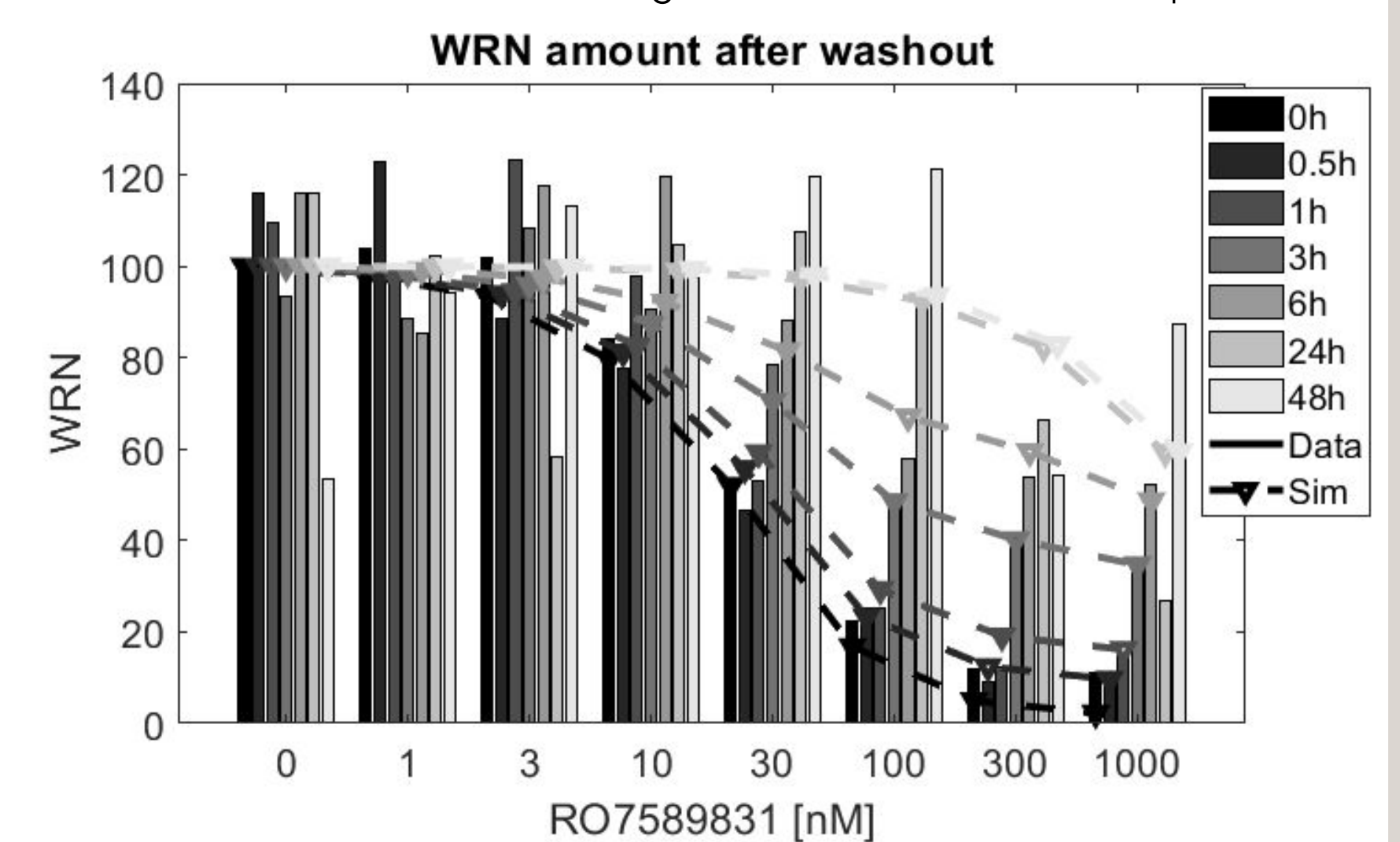


Figure 3. Fitting of simulated TE and experimental data of WRN helicase kinetics for different concentrations and at different timepoints after washout.

- Figure 4 illustrates how the mechanistic cell cycle model simultaneously captures target engagement dynamics, cell growth inhibition, and changes in cell cycle states with and without RO7589831 treatment. With 2μM WRNi, cells accumulate in the G2 phase, compared to the untreated condition, where cell cycle phases remain stable. Similarly, cell growth inhibition and a rapid decrease in WRN helicase levels are observed with 2 μM RO7589831.

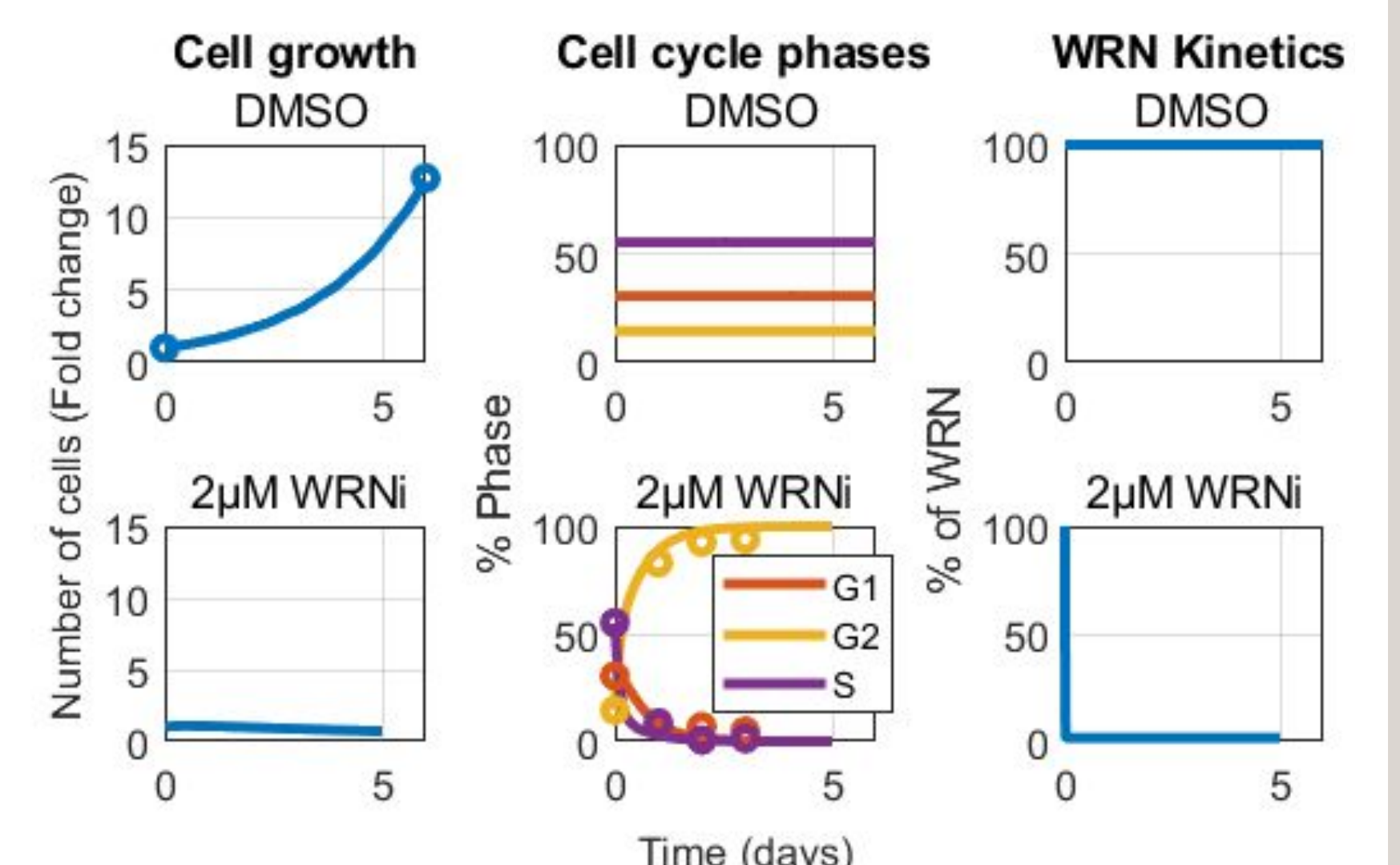


Figure 4. Time series simulations of cell growth, cell cycle phases and WRN kinetics with and without treatment (DMSO). The dots represent experimental data.

- Figure 5 demonstrates how cell viability simulations using the cell cycle model align well with experimental data across WRNi concentrations (1-10,000 nM) and washout periods, achieving a median rRMSE of 7.49%.

These results highlight the mechanistic model's capability to reproduce cell growth inhibition for various concentrations of RO7589831, driven by target engagement dynamics and their effects on the cell cycle.

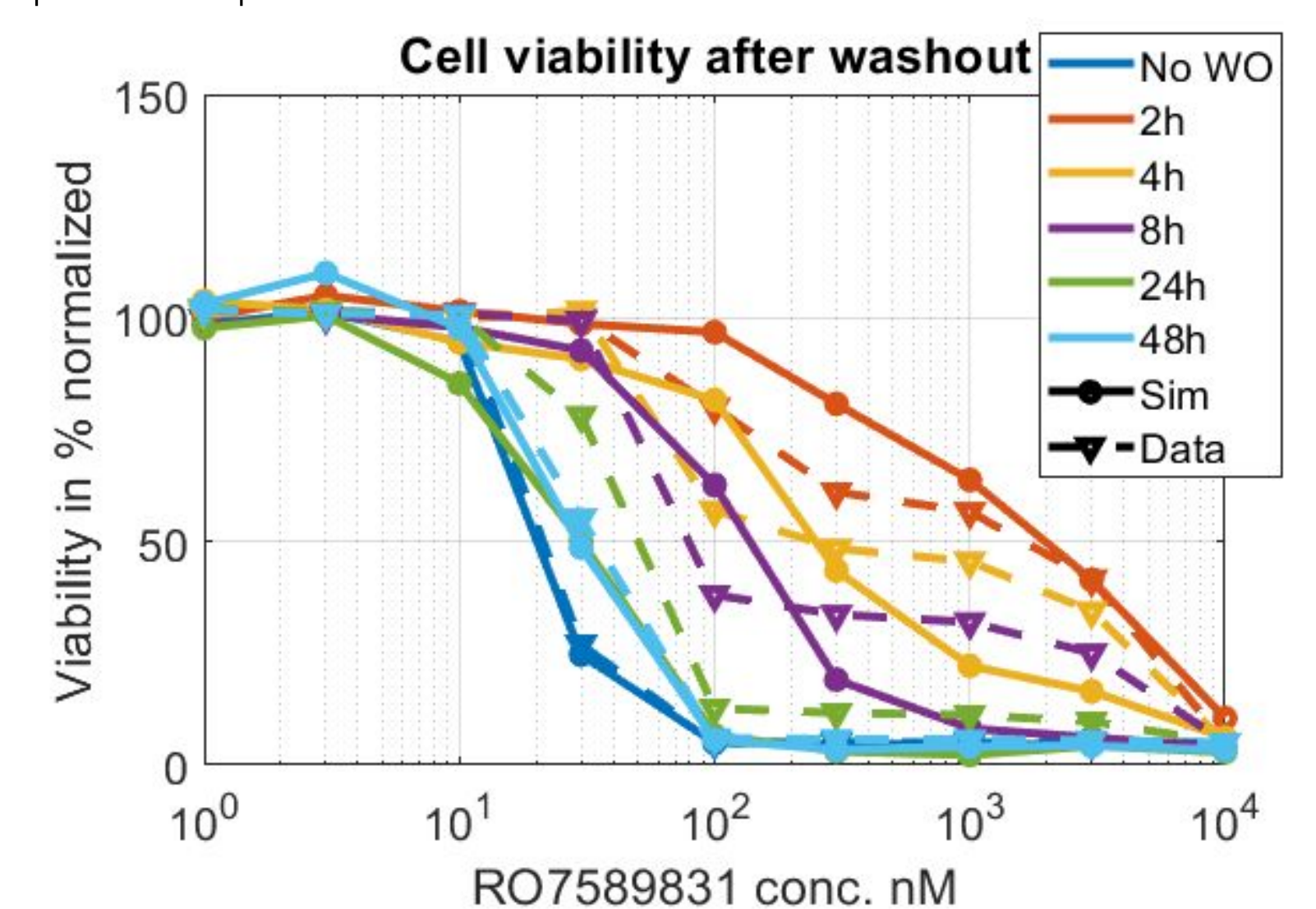


Figure 5. Goodness of fit of the cell cycle model of Cell Viability for different concentrations and different washout starting point. Cell viability measured at day 6.

Conclusions

- The developed mechanistic cell cycle model, integrated with a covalent target engagement model accurately replicates WRN target engagement and cell growth inhibition observed in MSI HCT116 cells treated with RO7589831.
- It provides a quantitative framework to explore variability in cell growth inhibition across different MSI cell lines, and establishes a link between WRN target engagement and cell growth inhibition.
- Further, it supports hypothesis generation for combination treatments with cell cycle-targeted agents.
- Potential future applications include:
 - Expanding the model for *in vivo* efficacy predictions.
 - Testing combination therapies predicted to enhance therapeutic outcomes.

References

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