

Modeling evaluation of different *in vitro* experimental designs to characterise antitumor drug effect

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Background & Objectives

Several pharmacodynamic models describing *in vivo* tumor growth have been reported in literature. However, relatively few models exist for *in vitro* cell growth [1-3]. This is partly due to current experimental protocols being often designed for empirical analyses. Here we evaluate two alternative *in vitro* study designs for developing (semi)mechanistic cell growth models to predict the antitumor effect of Oxaliplatin (L-OHP).

Methods – experimental designs

S1: Continuous L-OHP exposure

"Typical *in vitro* study"

- 10^4 SW480 cells/well
- 4 ≠ [LOH-P] (0.1, 1, 5 & $10\mu\text{M}$)
- 3 ≠ exposure times (24,48,72h)
- 4 plates, 1 measurement/well/plate

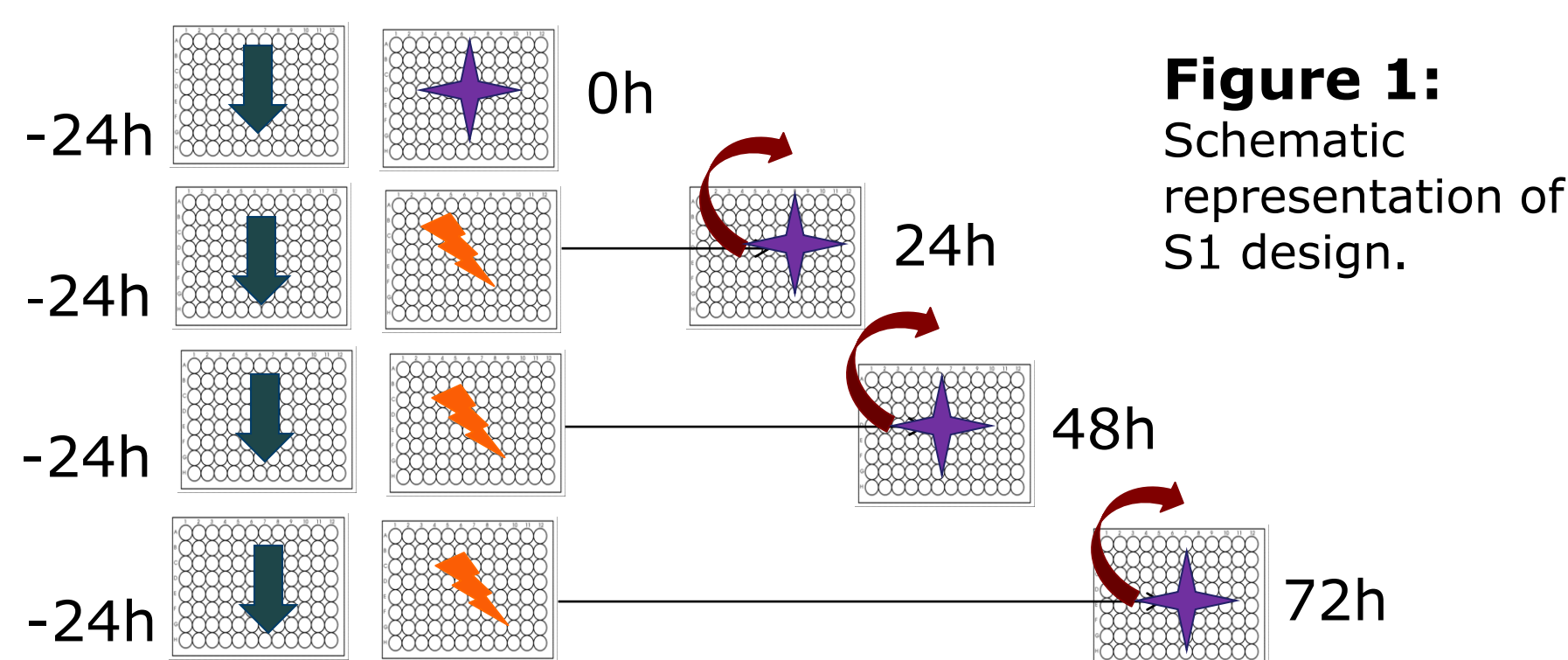
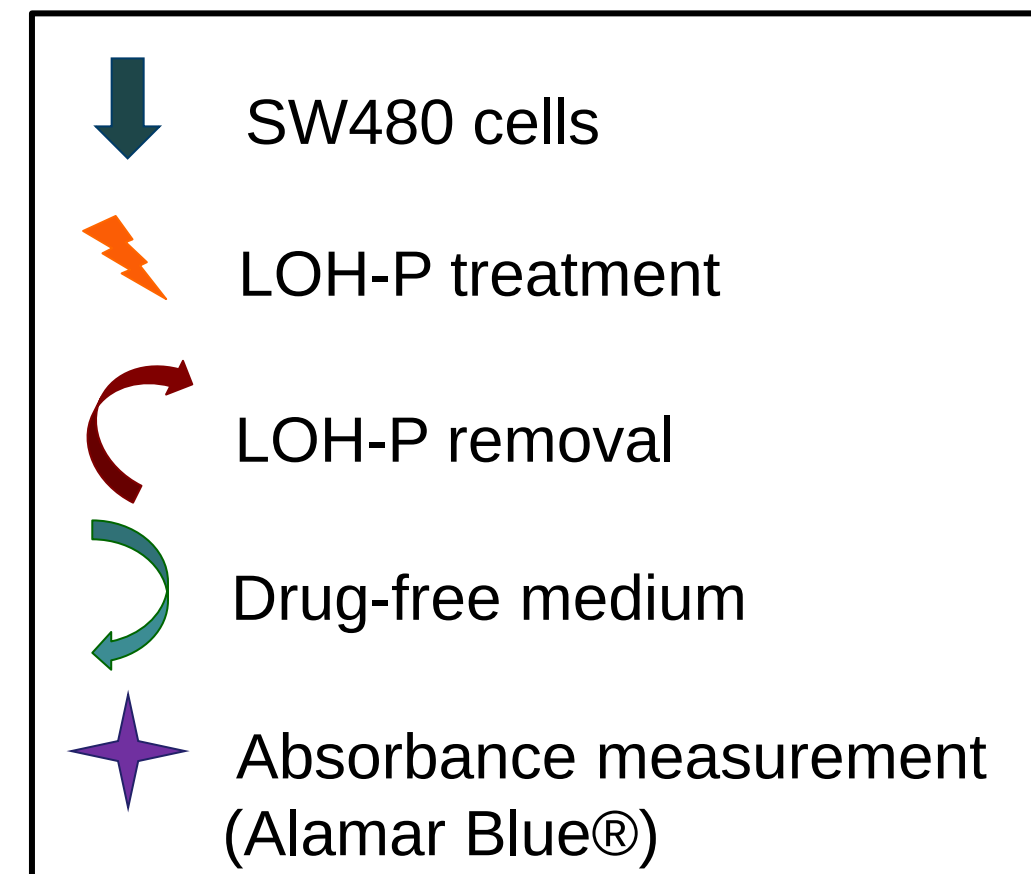


Figure 1: Schematic representation of S1 design.



S2: Non continuous L-OHP exposure

"New proposal design"

- 10^3 SW480 cells/well
- 3 ≠ [LOH-P] (0.1, 1, 5 or $10\mu\text{M}$)
- 2 ≠ exposure times (4 & 24h)
- 3 plates, 6-7 measurements/well/plate

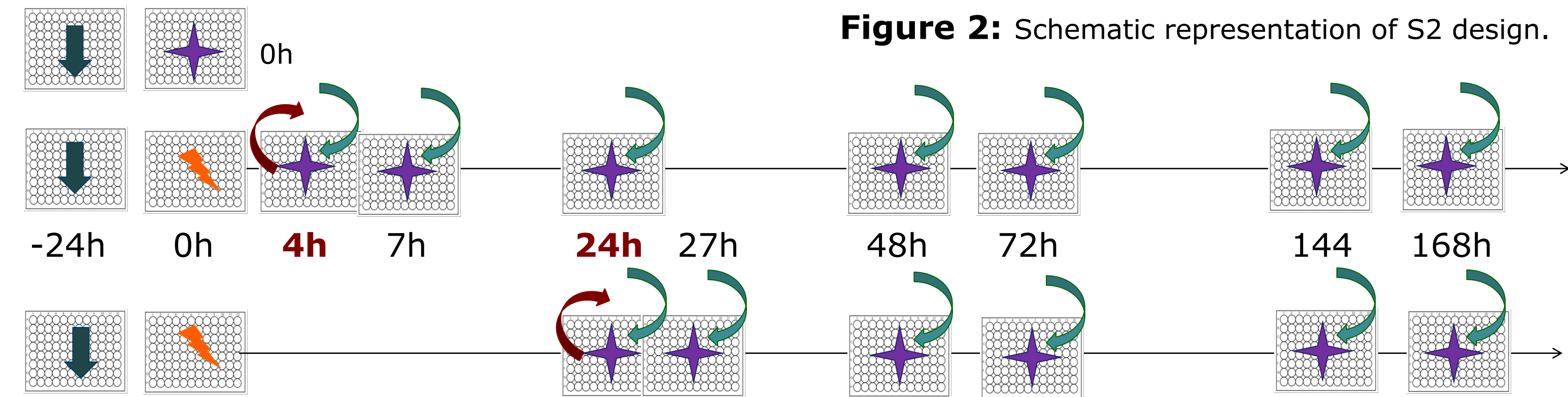


Figure 2: Schematic representation of S2 design.

Methods – analysis strategy

STEP 1

Dataset creation:
conversion of
absorbance
measurements to
cell count

Unperturbed growth:
≠ models tested:
lineal, exponential,
Gompertz, ...

STEP 2

Development of
a PD model for
each study
dataset

STEP 3

External validation:
model parameters of
each study used to
simulate the other one

STEP 4

Development of a
PD model
integrating data
from both studies

Further steps

- Integration of other studies
- Repeated doses
- ≠ cell lines & ≠ drugs

Results

STEP 1: The Gompertz model was used to describe the proliferation of SW480 cells

STEP 2a - BASIC MODEL: The antitumor effect was described by:

- Series of first order transit compartments to describe the delay.
- Lineal inhibition of the proliferation rate.
- Non-linear induction of cell death.

This model was developed independently for each study design. Both models described successfully their own study data (Fig. 4).

$$\frac{dCELLS(t)}{dt} = K_{PROL} \times (1 - SLP \times LOHP_4(t)) \times \log\left(\frac{N_{MAX}}{CELLS(t)}\right) \times CELLS(t) - \left(\frac{E_{MAX} \times LOHP_4(t)}{LOHP_4(t) + EC_{50}}\right) \times CELLS(t)$$

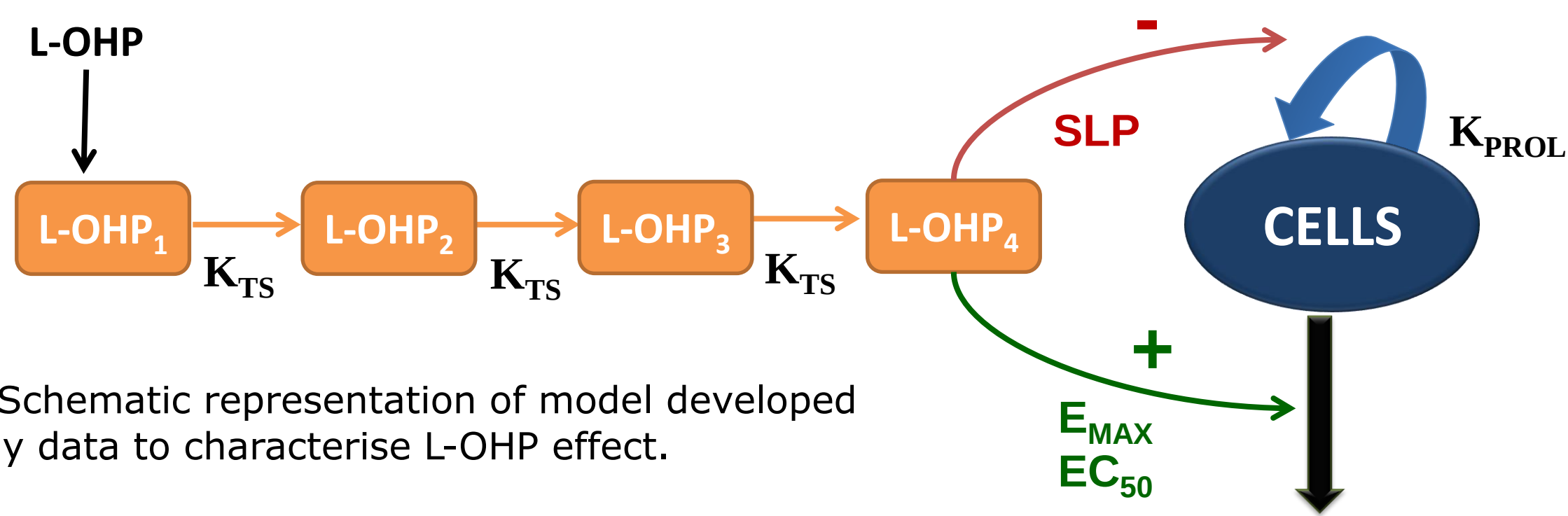


Figure 3: Schematic representation of model developed for each study data to characterise L-OHP effect.

STEP 2b: MODEL PREDICTIONS

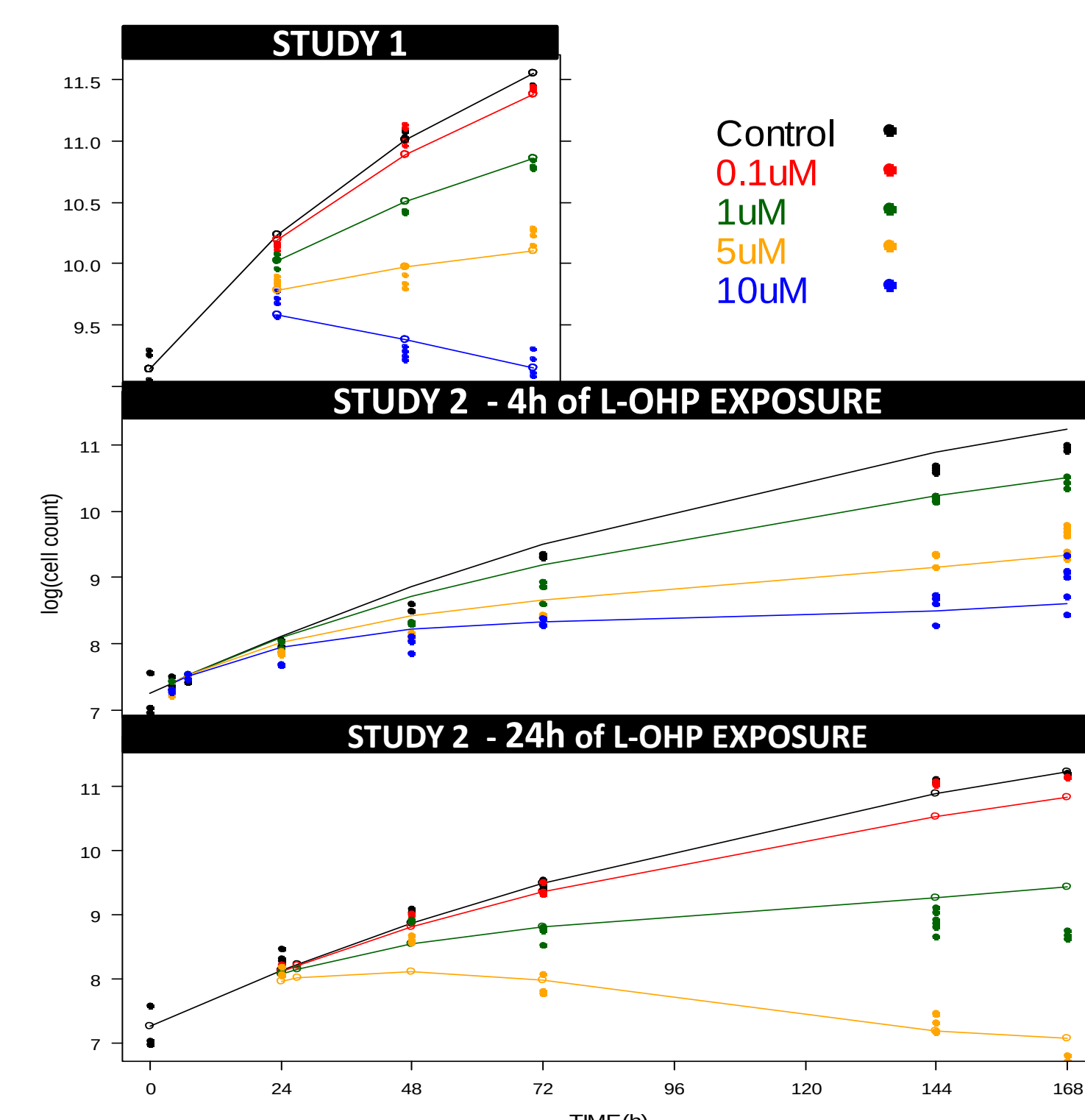


Figure 4: Observed cell count for each study (dots) and model predictions (lines) obtained with the basic model.

STEP 3: EXTERNAL VALIDATION

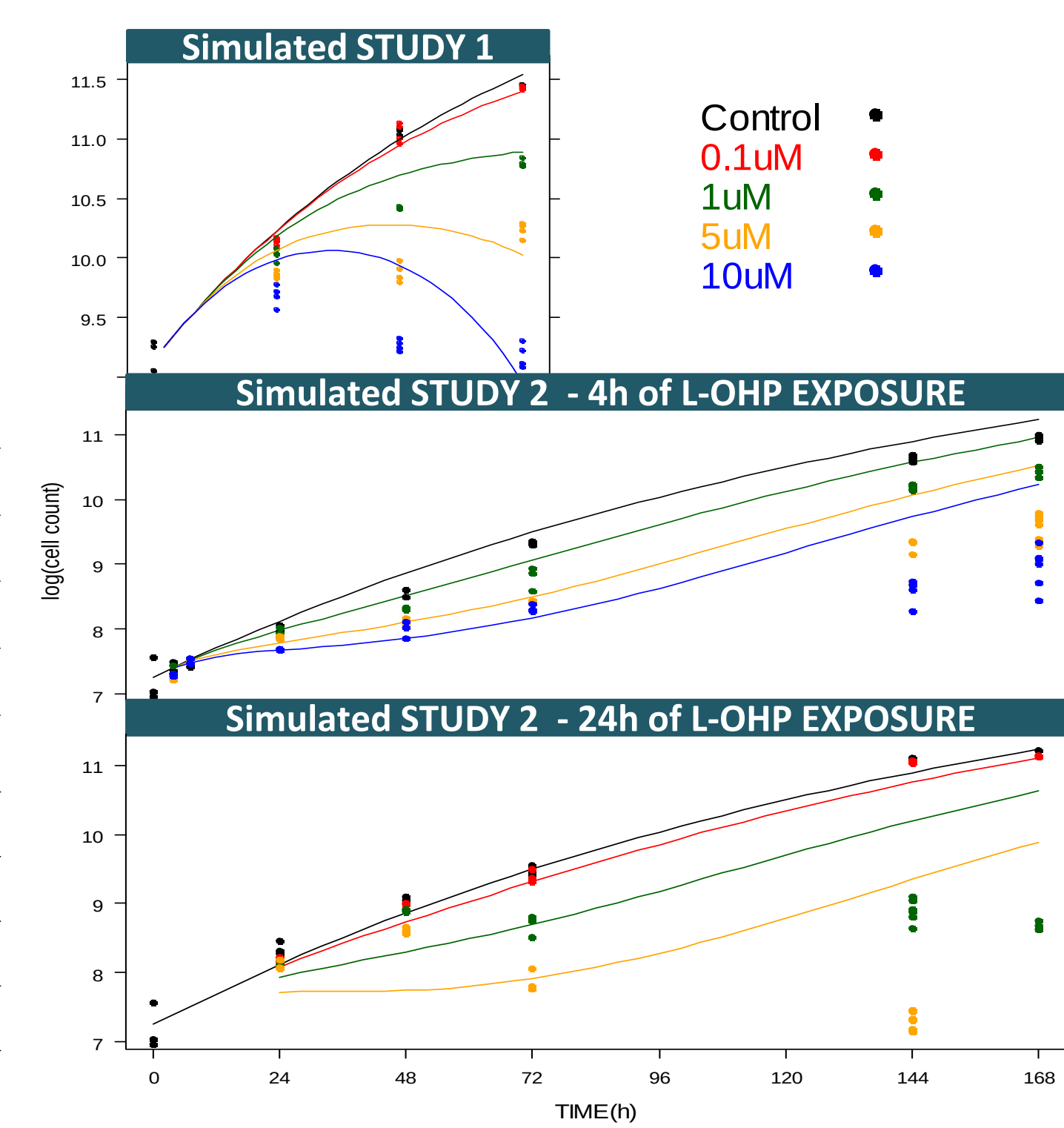


Figure 5: Observed cell count (dots) and simulated cell count (lines) obtained using the estimated parameters from the other study.

STEP 4: INTEGRATED APPROACH:

The magnitude of the antitumor effect was time and concentration dependent.

The model assumed:

- In each cell, drug and DNA interaction followed receptor-ligand(irreversible) binding kinetics to initial apoptosis.
- Transit compartments modeling the drug uptake and apoptosis cascade (Adduct1-Adduct2-Apoptotic cells) were used to explain the delay in the L-OHP effect.

This model is consistent with the previously reported mechanism of L-OHP [4].

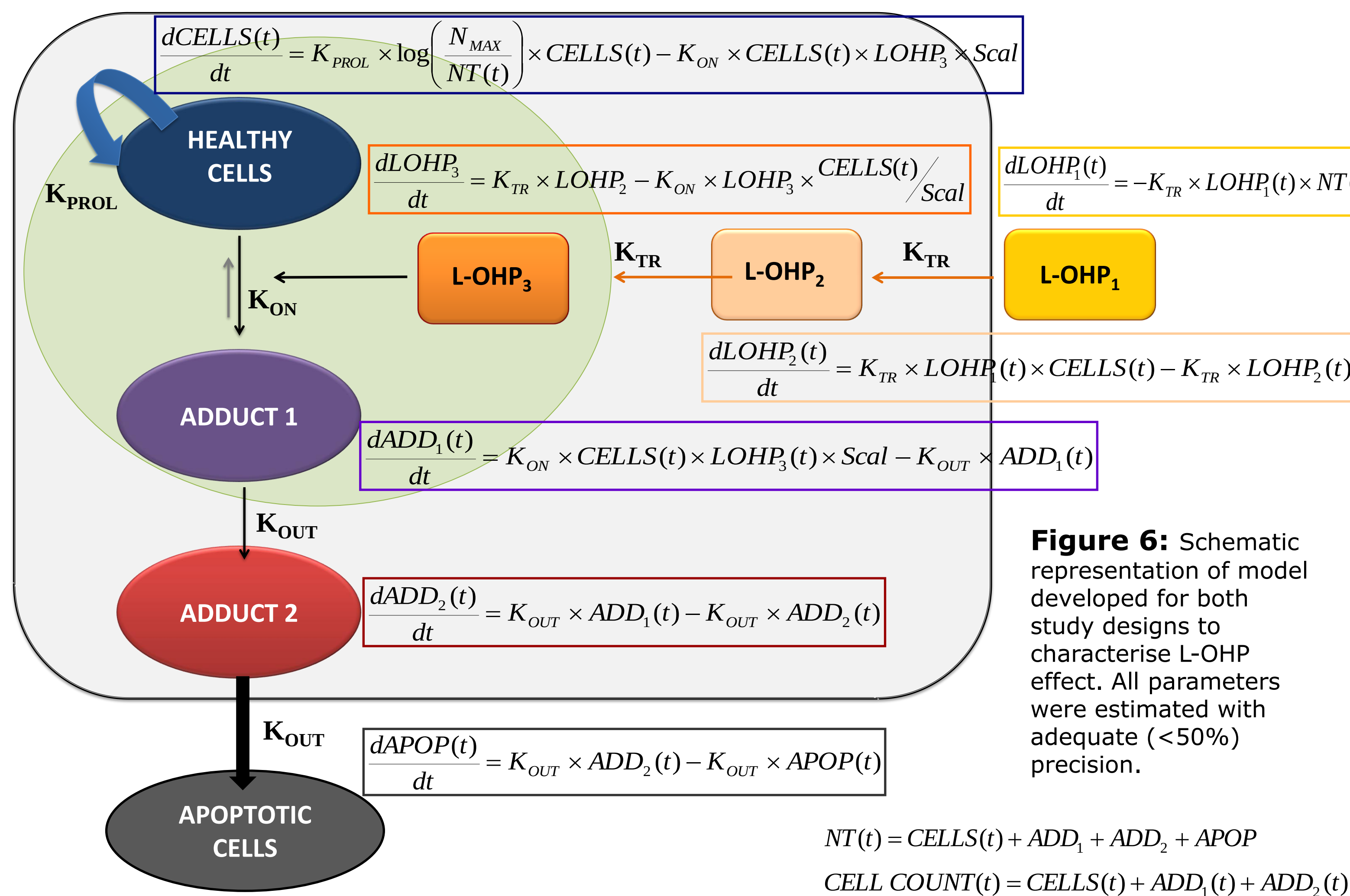


Figure 6: Schematic representation of model developed for both study designs to characterise L-OHP effect. All parameters were estimated with adequate (<50%) precision.

$$NT(t) = CELLS(t) + ADD_1 + ADD_2 + APOP$$

$$CELL COUNT(t) = CELLS(t) + ADD_1(t) + ADD_2(t)$$

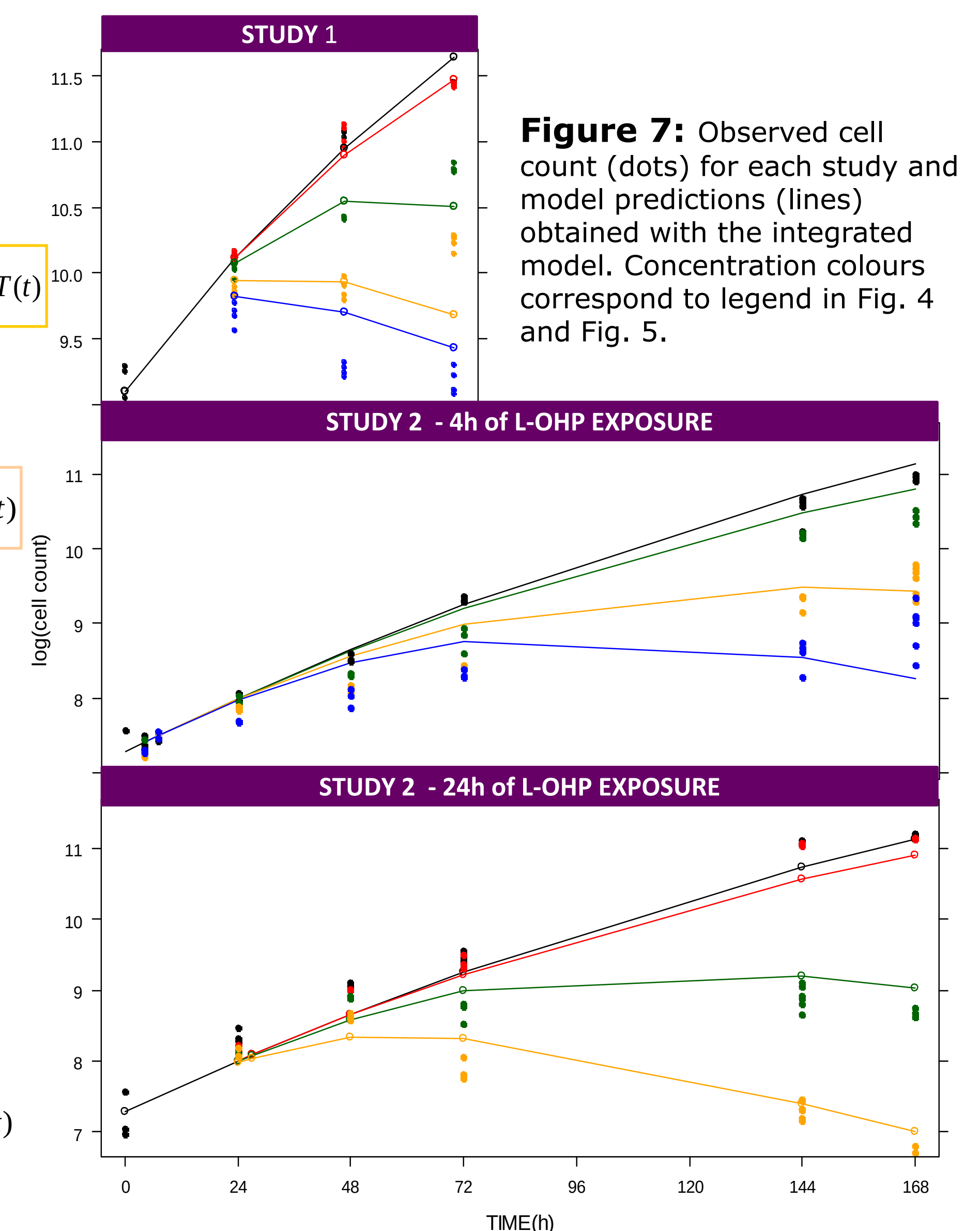


Figure 7: Observed cell count (dots) for each study and model predictions (lines) obtained with the integrated model. Concentration colours correspond to legend in Fig. 4 and Fig. 5.

Conclusions

- ✓ The best predictive performance was obtained with S2 design. Therefore, to facilitate the estimation of predictive (semi)mechanistic tumor cell growth models, we recommend *in vitro* cell growth studies to be performed, if possible, according to the proposed design.
- ✓ Based on this model, an integrated analysis would further benefit from the collection of specific biomarkers and drug concentrations to eliminate the need for empirical transit compartments.

References

- (1) Del Bene F et al *Cancer Chemother Pharmacol* 2009 Apr;63(5):827-836.
- (2) Lobo&Balthasar *The AAPS Journal* 2002;4(4):212-222.
- (3) Moreno D et al. *J Pharm Pharmacol* 2008;60(1):77-82.
- (4) Raymond et al *Mol Cancer Ther* 2002;1:227-235