

Predicting and optimizing drug combinations

There is currently a great deal of interest in identifying synergistic drug combinations. However, it is not easy to determine which schedules should be tested, since the number of different possible schedules increases combinatorially when more than one drug is considered. We have therefore developed a predictive PK-PD 'Virtual Tumour' model that allows rational design of schedules for drug combinations [1].

We previously built a Virtual Tumour™ that was capable of successfully simulating the outcome of various drug combination schedules in xenografts; using this model we were also able to propose new optimal administration schemas. Although xenografts represent a convenient and relatively inexpensive approach to assessing the likely efficacy of proposed dosing regimens *in vivo*, the number of permutations that can be tested is still limited by practical considerations.

We are therefore exploring the use of three-dimensional tumour cell cultures (microtissues) as a more cost-effective alternative to xenografts for validating Virtual Tumour™ predictions. Here we present a microtissue Virtual Tumour™ that is analogous to our xenograft model, and some preliminary results that demonstrate the potential of microtissues for predicting drug synergies.

The Physiomics Virtual Tumour™ technology

The Virtual Tumour™ (Fig. 1) is a sophisticated computer model that simulates tumour cell division and the effect of antineoplastic drugs, taking into consideration the differences between proliferative cells and those that are part of the necrotic core. The complexity of the model is deliberately constrained so that it can be parameterized with data that are usually produced during drug development. These data include PK data for the drug, biomarkers showing the cell population response, and xenograft growth measurements showing how tumour growth is affected. This technology provides a rationale for designing an appropriate schedule, and allows our partners to prioritize the most effective drug combinations.

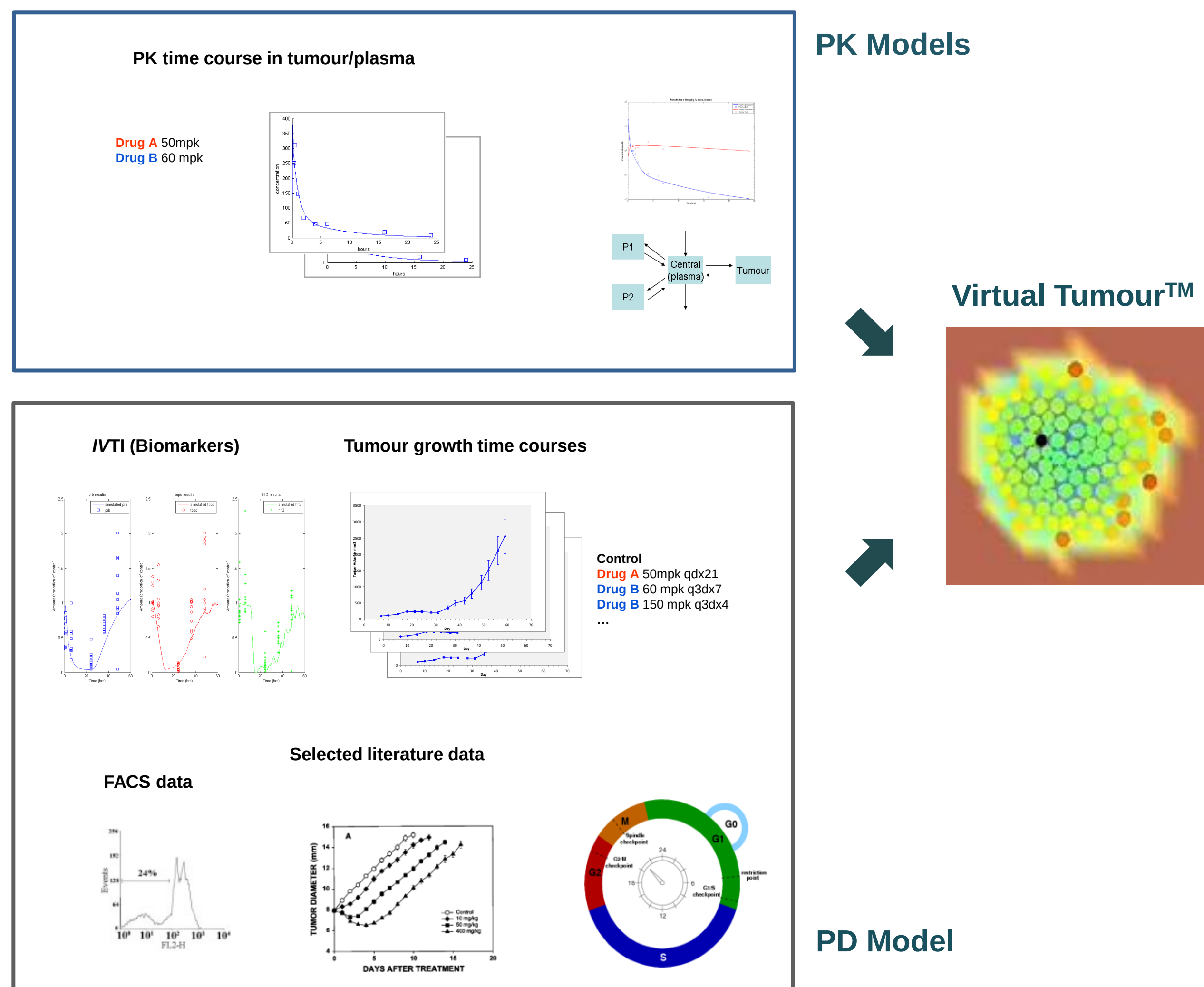


Fig. 1. The Physiomics Virtual Tumour™ simulation platform. The Virtual Tumour™ is a computer simulation of a growing tumour that integrates the cell division dynamic with the effect of antineoplastic agents. The platform is composed of PK models of the drugs of interest, as well as a pharmacodynamic model of cell cycle progression. Drug effect can be calibrated by using various data sources: *in vivo* target inhibition (IVTI), xenograft growth time courses, flow cytometry and public literature data.

Dose response and drug synergy in microtissues

In order to carry out a comparison with our previous study, in which gemcitabine-docetaxel combinations were optimized in MX-1 xenografts by applying the Virtual Tumour™ technology [2], we are currently developing an *in vitro* MX-1 microtissue model in collaboration with InSphero AG, as described previously [3].

MX-1:NIH3T3 microtissues were cultured as described and treated with a range of concentrations of gemcitabine and docetaxel, both alone and in combination. Microtissues were exposed for a fixed time to specified concentrations of a single drug, followed by medium exchange. In a preliminary combination study, microtissues were exposed to gemcitabine and docetaxel in sequence, separated by medium exchange. In each case there were two treatment periods, at 7 and 14 days after the start of cultivation. Microtissue growth, relative to untreated controls, was assessed at days 7, 14 and 21.

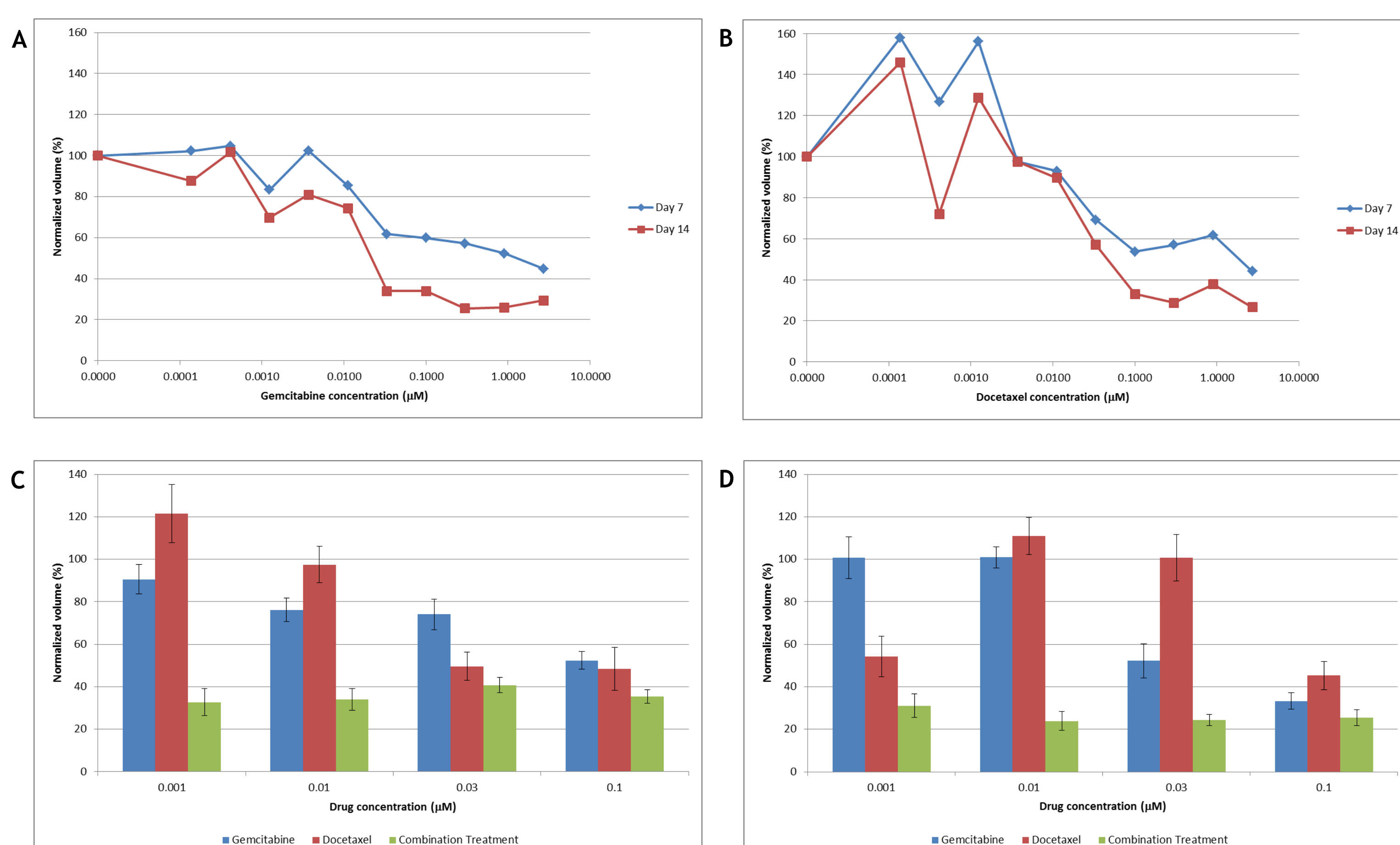


Fig. 2. A and B, dose response of MX-1:NIH3T3 microtissues to gemcitabine (A) and docetaxel (B), at 7 and 14 days post treatment; C and D, dose response of MX-1:NIH3T3 microtissues to gemcitabine and docetaxel alone and in combination, at 7 (C) and 14 (D) days post treatment, demonstrating a synergistic but saturable response to the combination.

Modelling microtissues with the Virtual Tumour™

The Virtual Tumour™ model used previously to simulate MX-1 tumour xenografts [2] was successfully adapted to simulate homotypic and heterotypic microtissue growth and response to drug treatment.

The model reproduces the dose-response to gemcitabine and docetaxel alone and in combination with a reasonable degree of accuracy - demonstrating the same saturable synergy between the drugs - and can also be used to explain the mechanism of this synergy.

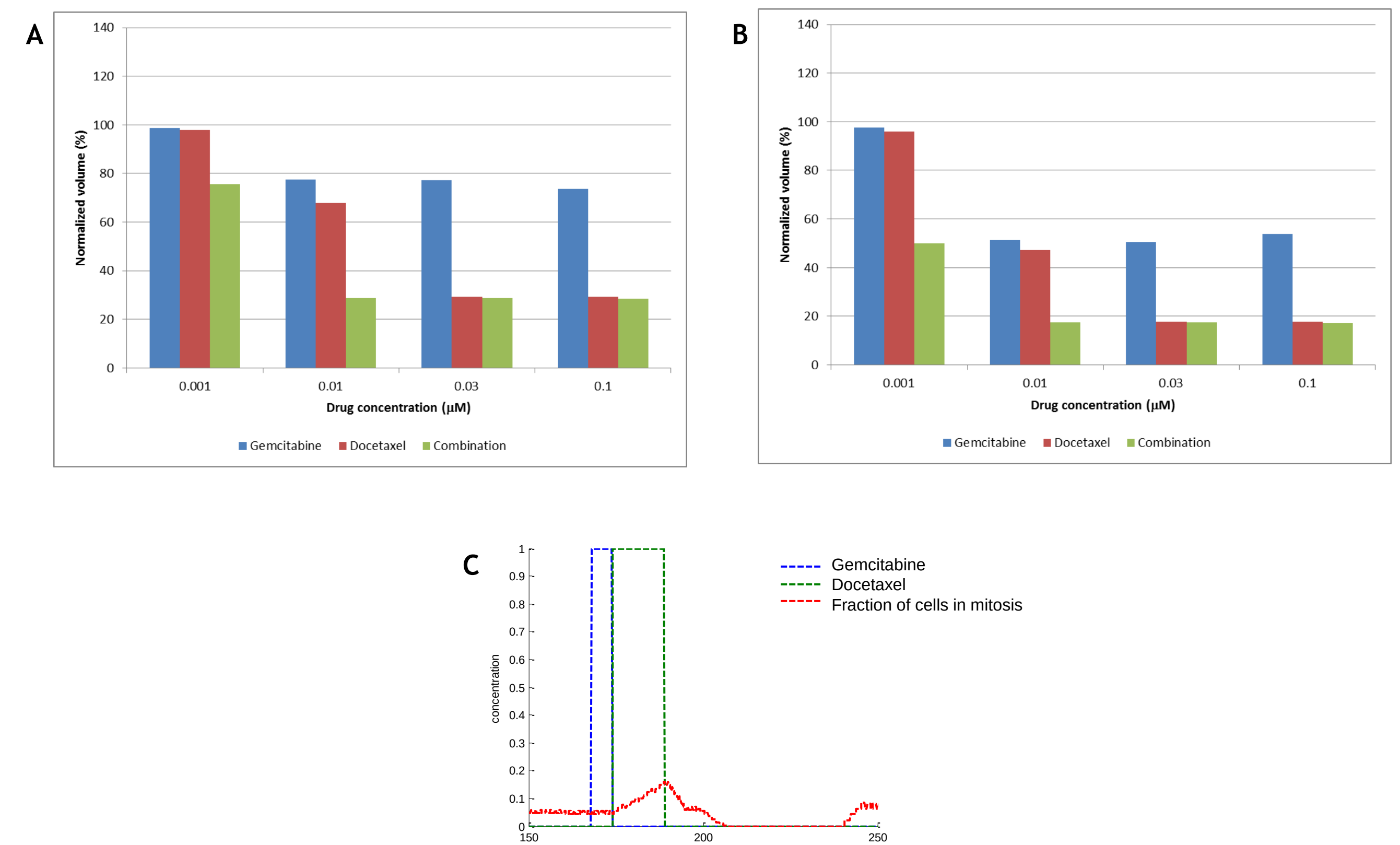


Fig. 3. Virtual Tumour™ simulation of microtissue growth and dose response to gemcitabine and docetaxel. Simulated dose response of MX-1:NIH3T3 microtissues to gemcitabine (A) and docetaxel (B), at 7 and 14 days post treatment; C, model output illustrating the synergy between the drugs, whereby the cytostatic effect of gemcitabine synchronizes cells and increases the proportion in mitosis, while docetaxel acts primarily on these cells to promote apoptosis.

Extrapolating from microtissue to xenograft

The Virtual Tumour™ microtissue model reproduces the experimental dose-response of A549 microtissues to MM-121 reported by Kalra *et al.* [4], as shown in Fig. 4A.

Extrapolation of the results of a dose-ranging study of MM-121 in A549 xenografts grown s.c. in nude mice [4] from the microtissue dose response, using the Virtual Tumour™, suggests that it could be feasible to predict the *in vivo* response from *in vitro* microtissue data (Fig. 4B).

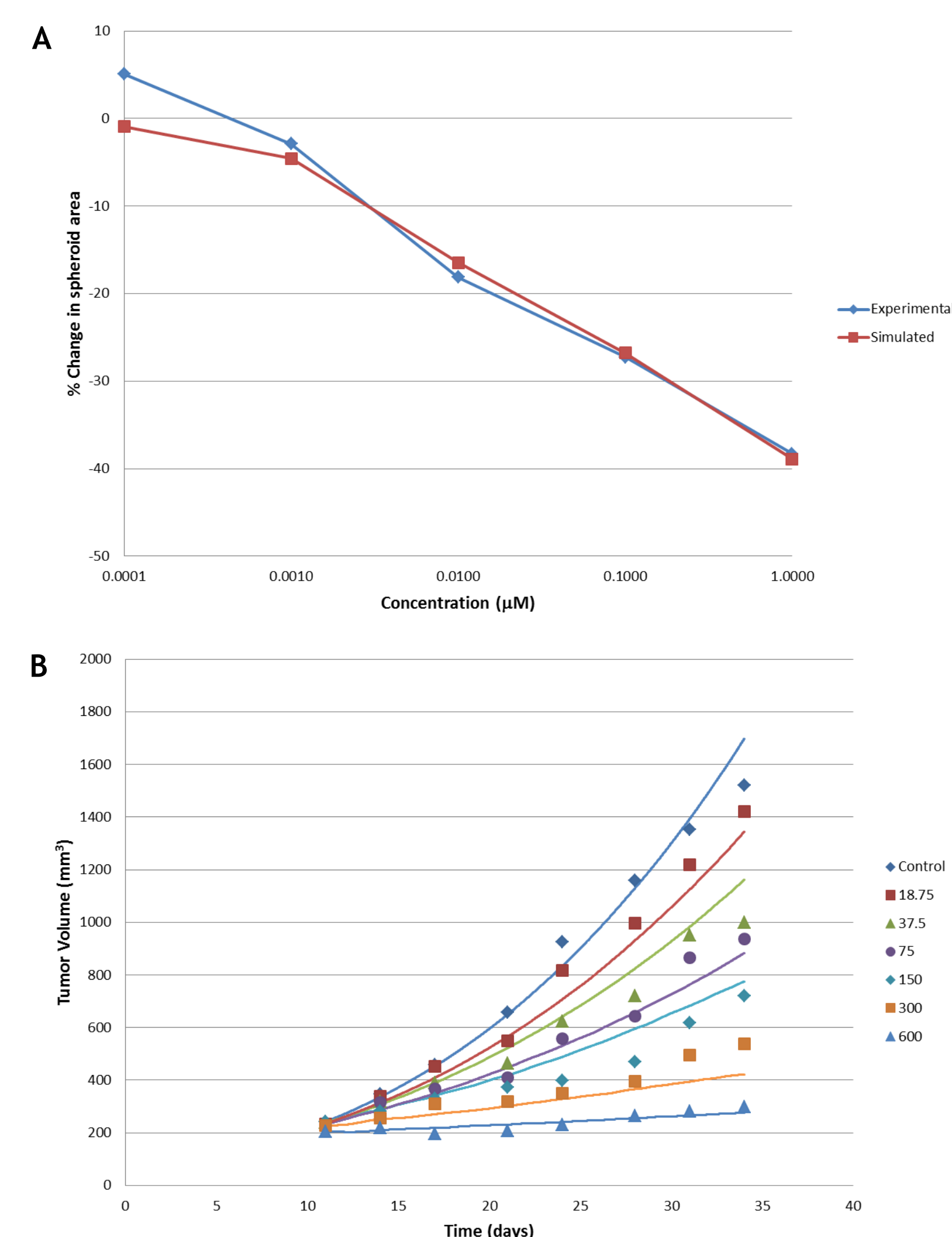


Fig. 4. Virtual Tumour™ simulation of microtissue and xenograft experimental dose-response studies with MM-121: A, Experimental [4] and simulated dose-response of A549 microtissues to MM-121; B, Experimental [4] and simulated results of a dose-ranging study of MM-121 in A549 xenografts.

Conclusion

In our preliminary work exploring the application of microtissues to validation of the Virtual Tumour™ technology we have developed an *in vitro* microtissue model for MX-1 in collaboration with InSphero AG, and adapted our existing xenograft Virtual Tumour™ to simulate microtissue growth and responses to antineoplastic agents. We have also successfully demonstrated a dose-dependent response to two such anti-cancer drugs, and have shown that the microtissues indicate a synergistic effect when the two drugs are dosed in combination.

This work suggests that *in vitro* microtissue data in combination with the Physiomics Virtual Tumour™ platform could be used to design new regimens and help test possible schedules with proprietary compounds as well as standards of care, small molecules or biotherapeutic agents, and allow prioritisation of the most effective drug combinations.

The predictive capabilities of this approach, including extrapolation from microtissues to xenografts, will be fully validated upon completion of a more extensive gemcitabine-docetaxel combination treatment study in MX-1 microtissues.

We thank InSphero AG for the experimental work.

References:

- [1] D. Orrell and E. Fernandez, Using Predictive Mathematical Models to Optimise the Scheduling of Anti-Cancer Drugs, *Innovations in Pharmaceutical Technology*, p59-62, June 2010.
- [2] Fernandez E. *et al.* (2011), Modeling the sequence-sensitive gemcitabine-docetaxel combination using the Virtual Tumor. AACR 102nd Annual Meeting, Orlando, FL, April 2-6, 2011.
- [3] Brightman, F. *et al.* (2011), The application of three-dimensional cell cultures in combination with the Virtual Tumor™ for designing optimal drug dosing schedules. EORTC-AACR Meeting, San Francisco, CA, November 12-16, 2011.
- [4] Kalra *et al.* (2009), MM-121, a first in class anti-ErbB3 antibody, shows efficacy in preclinical models of lung cancer: A potentially new treatment modality for human lung cancer. World Lung Conference, Cancun, Mexico, December 3-7, 2009.