The application of three-dimensional cell cultures in combination with the Virtual Tumor™ for designing optimal drug dosing schedules

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Predicting and optimizing drug combinations

There is currently a great deal of interest in determining 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 Tumor' model that allows rational design of schedules for drug combinations [1].

We previously built a Virtual Tumor™ 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 tumor cell cultures (microtissues) as a more cost-effective alternative to xenografts for validating Virtual Tumor™ predictions. Here we present a microtissue Virtual Tumor™ that is analogous to our xenograft model, and discuss the potential utility of this model in simulating and optimising drug dosing schedules.

The Physiomics Virtual Tumor™ technology

The Virtual Tumor™ (Fig. 1) is a sophisticated computer model that simulates tumor 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 tumor growth is affected. This technology provides a rationale for designing an appropriate schedule, and allows our partners to prioritize the most effective drug combinations.

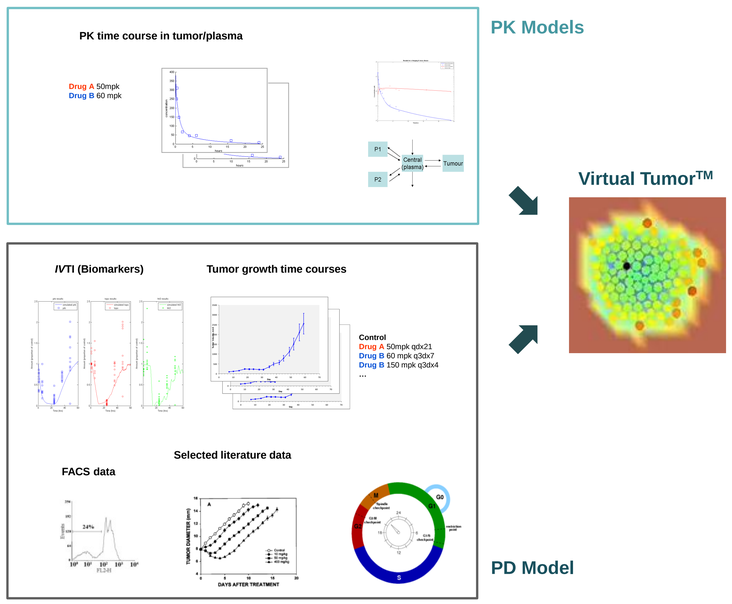

Fig. 1. The Physiomics Virtual Tumor™ simulation platform. The Virtual Tumor™ is a computer simulation of a growing tumor, which integrates the cell division dynamic with the effect of antineoplastic agents. The platform is composed of PK models of the drugs of interest, as well as a pharmacodynamic 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.

Microtissue formation

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 Tumor™ technology [2], we are currently developing an *in vitro* MX-1 microtissue model in collaboration with InSphero AG.

MX-1:NIH3T3 microtissues were produced in 96-well hanging GravityPLUS™ plates (InSphero AG, Zurich, Switzerland). Upon completion of the tissue formation process (4-6 days), microtissues were transferred into GravityTRAP™ microtissue assay plates.

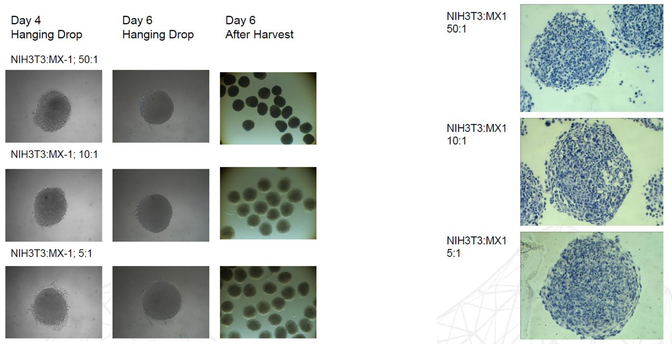

Fig. 2. Co-culture of MX-1 with NIH3T3 fibroblasts: A, formation of heterotypic microtissues with different starting ratios of NIH3T3:MX-1; **B**, morphological characterization.

Modeling microtissue growth with the Virtual Tumor™

The Virtual Tumor™ model used previously to simulate MX-1 tumor xenografts [2] was successfully adapted to simulate homotypic and heterotypic microtissue growth.

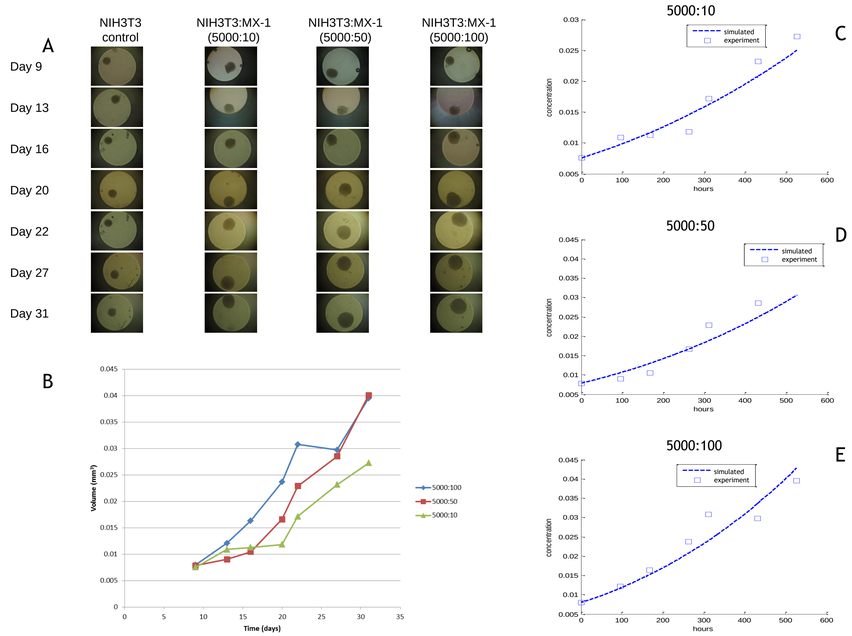

Fig. 4. Virtual Tumor™ simulation of microtissue growth: A and **B**, Experimental growth time courses; **C**, **D** and **E**, Overlay of simulated and experimental growth time courses.

Extrapolating from microtissue to xenograft

The Virtual Tumor™ microtissue model reproduces the experimental dose-response of A549 microtissues to MM-121 reported by Kalra *et al*. [3], as shown in Fig. 5A.

Extrapolation of the results of a dose-ranging study of MM-121 in A549 xenografts grown s.c. in nude mice [3] from the microtissue dose response, using the Virtual Tumor™, suggests that it could be feasible to predict the *in vivo* response from *in vitro* microtissue data (Fig. 5B).

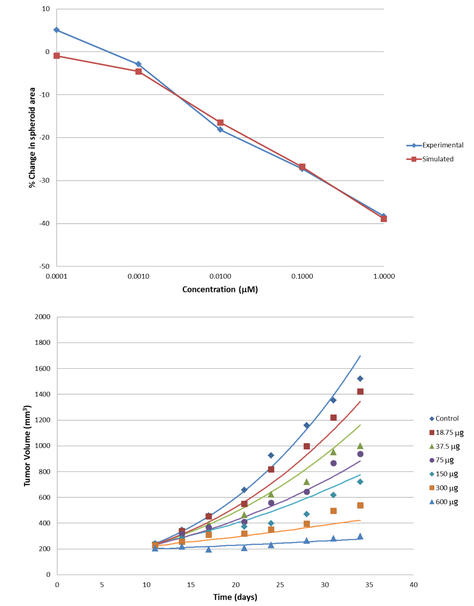

Fig. 5. Virtual Tumor™ simulation of microtissue and xenograft experimental dose-response studies with MM-121: A, Experimental [3] and simulated dose-response of A549 microtissues to MM-121; **B**, Experimental [3] and simulated results of a dose-ranging study of MM-121 in A549 xenografts.

Conclusion

In our preliminary work exploring the application of microtissues to validation of the Virtual Tumor™ technology we have developed an *in vitro* microtissue model for MX-1 in collaboration with InSphero AG, adapted our existing xenograft Virtual Tumor™ to simulate microtissue growth and responses to an antineoplastic agent, and successfully extrapolated from microtissue to xenograft.

This work suggests that *in vitro* microtissue data in combination with the Physiomics Virtual Tumor™ 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 biotherapeutical agents, and allow prioritisation of the most effective drug combinations. The predictive capabilities of this approach will be fully validated upon completion of a 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] 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.