Modeling the sequence-sensitive gemcitabine-docetaxel combination using the Virtual Tumor

Eric Fernandez[1], David Orrell[1], Caroline Mignard[2], Zina Koob[2], Damien France[2], Nicolas Hoffmann[2], Francis Bichat[2], David Fell[1], Christophe Chassagnole[1]

[1]Physiomics plc, The Oxford Science Park, Oxford, OX4 4GA, United Kingdom. Tel. +44 (0)1865 784980. Email: efernandez@physiomics-plc.com
[2]Oncodesign, 20 rue Jean Mazen, 21076 Dijon, France

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Predicting and optimizing drug combinations

In recent years there has been great interest in determining synergistic drug combinations. A difficulty, however, is that the number of different possible schedules increases combinatorially when more than one drug is considered, so it is very hard to know what schedules should be tested. Physiomics, a computational biology company based in Oxford UK, has therefore developed a predictive PK-PD "Virtual Tumour" model that allows us to rationally design schedules for drug combinations [1]. In this poster, we show how the Virtual Tumor was used to predict and optimize a gemcitabine-docetaxel combination.

The combination of fixed-dose-rate gemcitabine and docetaxel has become an established first- or second-line treatment option for many types of cancer [2-3]. Several clinical studies have attempted to improve efficacy of these two drugs by designing innovative gemcitabine-docetaxel sequences and schedules in various cancer types [reviewed by 4].

We have entered a collaboration with the French CRO Oncodesign to validate our model. By producing xenograft and biomarker data of each drug in isolation, we have built a Virtual Tumour capable of predicting the outcome of various regimens using this combination, and demonstrated how optimal administration schemas can be determined *in silico*.

Experimental methods

- **Test substances:**
  - docetaxel prepared in Ethanol/Polysorbate 80/saline (5/5/90)
  - gemcitabine prepared in saline
- **Tumor material:** human MX-1 breast carcinoma
- **Animals:** Swiss Nude mice (Charles River, France)
- **Tumor induction and route of drug administration:**
  - Sub-cutaneous implantation of MX-1 tumor fragment to Nude mice
  - Randomization of mice based on tumor volumes at 100-200 mm3 (or 300-400 mm3 for PK/PD study)
  - IV bolus injection of docetaxel through the tail vein
  - IP injection of gemcitabine
- **Monitoring:**
  - Daily monitoring of mouse survival
  - Twice a week monitoring of mouse body weight and tumour volumes

Physiomics Virtual Tumour technology

The 'Virtual Tumour' (Figure 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 parameterised with data that is usually produced during drug development. This data includes 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 prioritise the most effective drug combinations.

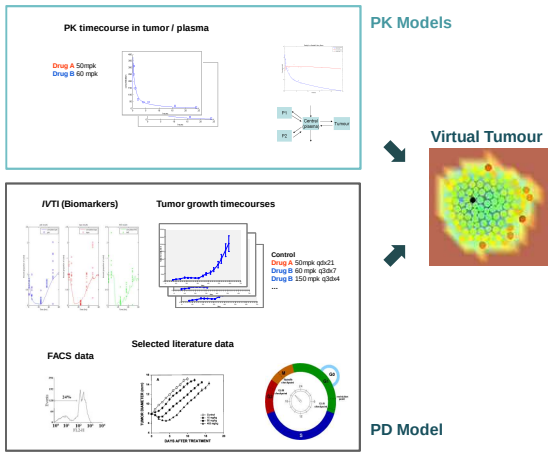

Fig. 1. Physiomics Virtual Tumour simulation platform. The Virtual Tumour is a computer simulation of a growing tumour, which integrates the cell division dynamic with the effect of antineoplastic agents. The platform is composed of PK models of the drugs of interest, as well as a pharmacodynamic model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

PK and PD study of Gemcitabine and Docetaxel for Virtual Tumor calibration

In order to accurately model drug effect on cell cycle progression and calibrate the Virtual Tumor, we generated experimental PD and PK data obtained from single drug injection. Tumor sample analysis was done at different time points after dosing (from 0 to 72 hours, 4 mice per time point).

Pharmacodynamic data

Figure 2 shows detection of phospho-histone H3 and topoisomerase II in O.C.T embedded tumor samples by immunohistochemistry. PD model was calibrated using these *IVTI* data: these helped understand the effect of each drug on MX-1 cell-cycle progression and calibrate the timing-related effect of each drug.

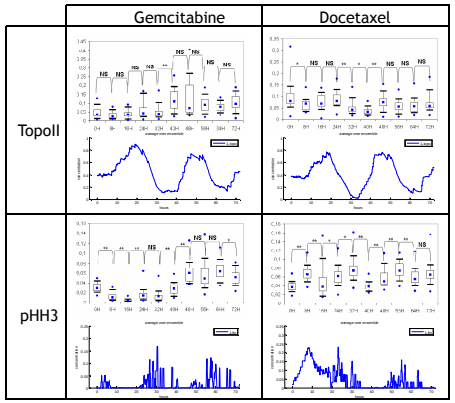

Fig. 2. Biomarker expression levels in treated tumour. Topoisomerase II and phospho-histone H3 *in vivo* target inhibition were measured in xenograft MX-1 tumors at 8-hour intervals after single Gemcitabine and Docetaxel injection. In each cell, top figure are the experimental measurements and bottom figure is a simulated time course after model calibration.

Pharmacokinetic data

Docetaxel and gemcitabine levels were determined in plasma and frozen tumor samples using HPLC/MS-MS method. Calibrated PK models for each drug were built for use in Virtual Tumor (Figure 3).

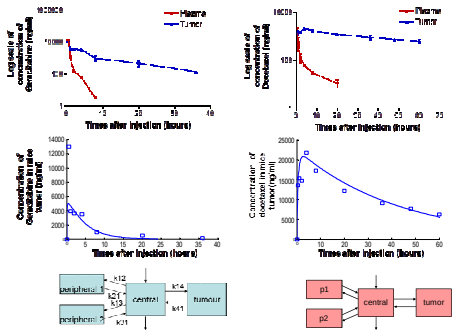

Fig. 3. Pharmacokinetic timecourse profiles. Top: PK profiles were determined for each drug in plasma and tumor after single injection. Middle: Simulated time courses (solid lines) are overlaid with experimental values in tumor (squares). Bottom: PK compartmental models built for each drug.

Predicting xenograft growth drug combination

The two drugs have different mechanisms of action, and lead to cell progression delay in different phases of the cell cycle. We therefore sought a schedule that would maximize the effect of the two drugs through cell synchronization, timing effects and PK time course profiles. In particular, we focused on the sequence and offset time between the two drug injections. By using the Virtual Tumor, it was possible to scan a high number of drug injection possibilities and predict which regimens could lead to the best synergy between the two drugs.

We identified 3 schedules that were of particular interest (Figure 4):

- one schedule expected to result in the worst synergy: Gemcitabine then Docetaxel 4 hours later;
- two schedules expected to result in better synergy: Gemcitabine then Docetaxel 12 hours apart and Docetaxel then Gemcitabine 10 hours apart.

Four cycles of drug administration were made for each regimen. Total drug dose was constant in all regimens (each dose: Gemcitabine 60 mg/kg, Docetaxel 7.5 mg/kg).

Drug sequence	Offset	Schedule	Figures 5-6 timecourses
Gemcitabine → Docetaxel	4 hours		
Gemcitabine → Docetaxel	12 hours		
Docetaxel → Gemcitabine	10 hours		

Fig. 4. Selected drug combination schedules. Gemcitabine (blue bars) and docetaxel (red) are administered every 7 days using specific injection offset in each case.

The three schedules were experimentally tested in xenograft models, along with no-drug or single-drug treatment controls (Figures 5 & 6).

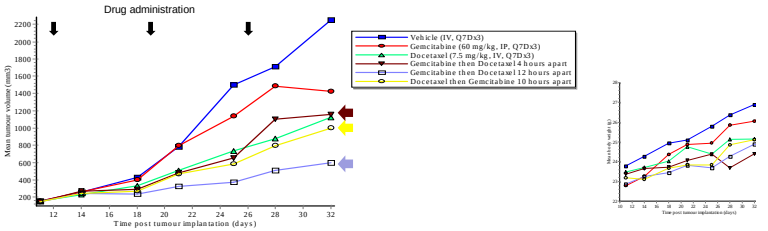

Fig. 5. Efficacy study of predicted combination regimens. The three schedules shown in Figure 4 are highlighted with corresponding arrows. Drugs were administered at day 12, 19 and 26. Right panel: mean body weight for each group.

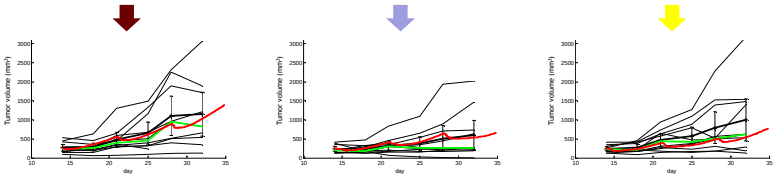

Fig. 6. Plots of tumor growth for predicted combination regimens. The three schedules shown in Figure 4 are identified by corresponding colored arrows. Individual tumor size (thin black line), mean tumor size (thick black line), median (green), prediction (red).

Virtual Tumor allowed us to predict a combination regimen ~50% more efficient than another predicted regimen that was lacking synergy. Furthermore, this level of efficacy could be obtained with a lower dose of docetaxel than typically used in xenograft studies (7.5 mg/kg vs 10 mg/kg). Finally, toxicity was not higher in the more efficient combinations, as shown in mean body weight plots. This shows how critical timing can be when administrating drugs having different mechanisms of action and how predictive models could be used for optimization.

Conclusion

We have demonstrated that the Virtual Tumor could be used to predict the outcome of various gemcitabine - docetaxel combination regimens and to optimize the delivery schedule. Using PD and PK data that can be gathered during the development of a drug, this could lead to dramatic improvement in its usage, efficacy and prevent loss of synergy due to timing issues.
This technology can be used to design new regimens with proprietary compounds as well as standard of care, small molecules or biotherapeutical agents; help test possible schedules for combinations of different drugs that would be effectively impossible to investigate experimentally; and allow prioritisation of the most effective drug combinations.

References:
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