BRIDGING GAPS IN TRANSLATIONAL BIOLOGY : Exploring pharmacokinetic/pharmacodynamic/efficacy relationships and combination treatments in 3D tumour models using a microphysiological system

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INTRODUCTION

Developing effective oncology therapies involves defining the right schedules to minimize side effects and maximise efficacy. This requires an accurate understanding of the pharmacokinetic/pharmacodynamics (PK-PD) relationship of the compound(s) (1). Animal and human PKs can differ significantly and many failures of novel therapies are due to a missing physiologically relevant link between preclinical and clinical data.

In vitro experiments are mainly performed at fixed concentrations and do not explore PK-PD relationships, which represents a limit to their translational relevance (2). To overcome this issue, we have developed a microphysiological system (MPS) able to explore PK/PD efficiency relationships on 3D tumour models/organoids, following mono or combination therapy.

AIMS

1. Demonstrate MPS is able to generate PK profiles for monotherapy and combinations oncology therapies.
2. Demonstrate MPS ability to mimic oncology treatment regimens on complex 3D tumoroids and assess treatment efficacy and PK/PD relationship.

MATERIALS AND METHODS

Experiments carried out using PhysioMimix-PK (PMX-PK) MPS device and controller (CN Bio Innovations), PK profiles were derived from literature data (3, 4) and adapted using MPS editor software.

Tumour cell lines used were A549 (non-small lung carcinoma, NSCLC) and SW620 (colorectal carcinoma) cultured as2D monolayers or 3D tumoroids encapsulated in Matrigel droplets to better mimic tumour microenvironment.

3D tumoroids were collected at 48h from seeding and transferred to 24-well plates, encapsulated in 4 µL Matrigel droplets (2 droplets per well). Plates were placed in the MPS device using a proprietary lid prior to microfluidic experimental onset.

At endpoints, cells were imaged using a Nikon Eclipse Ti2 inverted microscope and image analysis used customized macros in Fiji ImageJ (5). Cell viability was assessed using CellTiter-Glo® 3D Cell Viability assay (Promega). pAKT expression was assessed by AKT (Phospho) [pS473] Human ELISA kit (ThermoFisher).

RESULTS

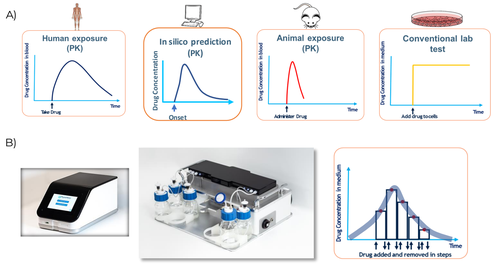


Figure 1 – MPS device mimics *in vivo* PK profiles
A) Comparing drug exposure profiles, typically in vitro lab tests do not recapitulate in vivo drug exposure. B) PhysioMimix-PK MPS controller and PK generator device forming a platform to treat 2D and 3D tumour models and is able to replicate/programme PK profiles using bespoke device software

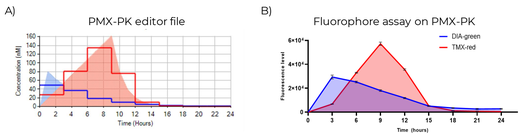


Figure 2 – MPS device recapitulates combination drug profiles
A) Snapshot of MPS software with PK profiles for two drugs added to culture wells across time. B) Measured PK profiles for 2 drug-equivalents (fluorescent probes) across 24h from multiple culture wells from MPS device. N = 3 ± SD.

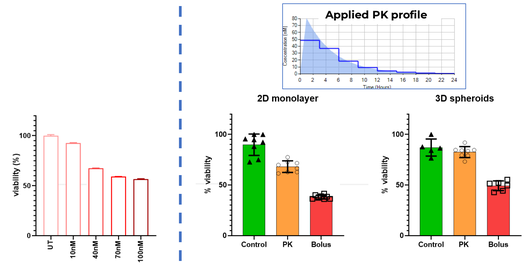
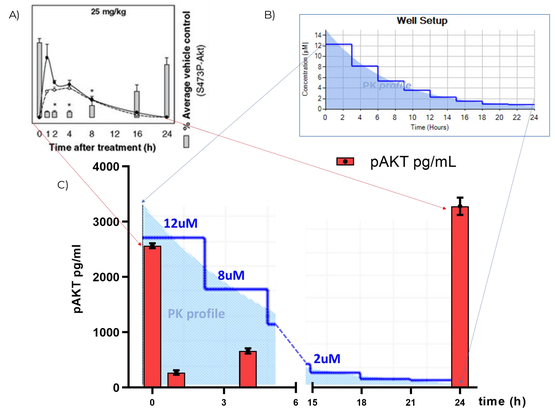
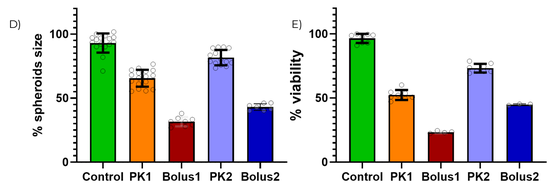


Figure 3 – Mimicking *in vivo* drug exposure in 3D tumoroids prevents overprediction of efficacy in traditional 2D cultures
SW620 colorectal cancer cell line was cultured as a monolayer (2D) and tumoroids (3D), then treated with topoisomerase inhibitor (SN38) for three days and cell viability assessed. Treatment followed either a PK profile mirroring murine *in vivo* exposure (equivalent to 500 mg/kg every 24 h for 72 h) or bolus exposure at 80 nM (continuous drug exposure – traditional *in vitro* approach). N = 3; mean ± SD.





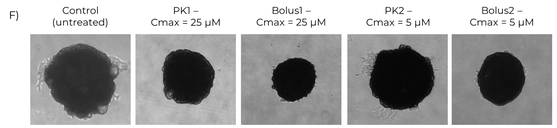


Figure 4 – Demonstrating PK/PD/efficacy relationship *in vitro* for a PI3Kα inhibitor treatment of lung tumours using MPS device.
PK/PD/efficacy relationship was assessed for PI3K inhibitor BYL719 treatment of NSCLC cells (A549). A) PK/PD relationship in mice for BYL719 and p-AKT in Rat1-myr-p110α tumours as determined by Fritsch *et al.* (3). B) BYL719 PK profile, applied to Matrigel encapsulated A549 tumoroids using MPS (equivalent to 50 mg/kg murine oral dose) for 24h – cellular samples taken at regular intervals to assess C) cellular p-AKT levels. D-F) Equivalent tumoroids were exposed to PK profile doses (or bolus continuous doses) of BYL719 daily for six days. PK1/bolus1 – 25 µM Cmax equivalent to 25 mg/kg murine oral dose and PK2/bolus2 Cmax equivalent to 12.5 mg/kg murine oral dose (taken from literature - 3). Tumours were assessed for D) spheroid size and E) viability (N = 3; mean ± SD). F) Representative images of 3D tumoroid model, post treatment.

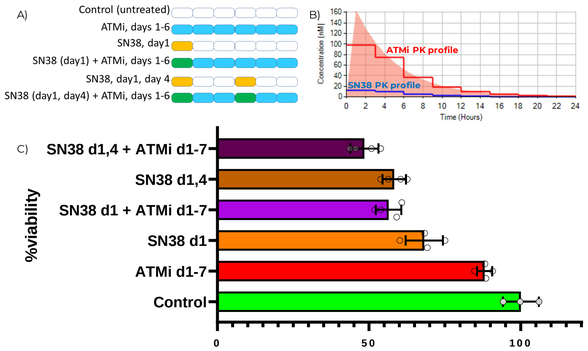


Figure 5 – Comparing combination drug dosing regimens for colorectal tumours *in vitro* using MPS device. SN38 (topoisomerase inhibitor) monotherapy and combinations with a DNA-damage response inhibitor (ATMi) efficacy on SW620 tumoroids were assessed using MPS device. A) SN38/ATMi treatment regimens were designed to recapitulate *in vivo* experiments (4). B) PK profiles for SN38 (Cmax = 20 nM) and ATMi (Cmax = 162 nM), applied on SW620 3D tumoroids, using our MPS, for 6 days were adapted from literature equivalents (SN38 Cmax equivalates 150 mg/kg murine oral dose of Irinotecan; ATMi Cmax equivalates 150 mg/kg murine oral dose). C) Endpoint viability for the SN38/ATMi monotherapy and combinations using each drug specific PK profile (displayed on B) and *in vivo* derived regimens. N = 3 mean ± SD.

CONCLUSIONS

Demonstrating PK/PD/efficacy relationships *in vitro* is typically not possible; using the developed MPS we were able to simply and accurately recapitulate *in vivo* PK profiles and expose 2D and 3D tumour models to various drug treatment regimens.

The translational relevance of our MPS was demonstrated by recapitulating dosing of BYL719 a PI3Kα inhibitor, with A549 NSCLC tumoroids and demonstrating dynamic effects on the biomarker p-AKT.

Drug combination and therapy scheduling was demonstrated using the MPS' ability to recapitulate *in vivo* dosing of topoisomerase inhibitor SN38 and DNA-damage response inhibitors on SW620 colorectal cancer tumoroids; results obtained were consistent with xenograft data (4). With greater throughput, simpler set up and improved translation (ability to compare human and animal PK profiles), we demonstrate the MPS can help enhance pre-clinical oncology studies.

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