In vitro assessment of combination dosing regimens with *in vivo* like pharmacokinetic concentration profiles enabled by a microfluidic addition and removal device

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Abstract No: 10816
Poster No: LB-044
Meeting: AACR 2018

INTRODUCTION

The pharmacokinetic (PK) profile is a determining factor in both the safety and efficacy of a drug or therapeutic regimen. PK profiles can vary significantly between patients and between humans and pre-clinical animal species.

Mouse Xenograft models are ubiquitous in oncology research, widely utilized to study single agents, combinations and scheduling. Differences in PK between mouse and human are one factor limiting the translational relevance of xenograft studies. Additionally, studies to assess combinations and/or scheduling quickly require large numbers of animals owing to the many possible permutations.

Here we describe a device capable of recapitulating PK-like profiles *in vitro*, and explore the effects of PK on the treatment of non-small-cell lung carcinoma by erlotinib alone and in combination with pemetrexed.

AIM

Utilise a microfluidic addition and removal platform to i) recapitulate PK profiles *in vitro*, ii) model combinations of erlotinib and pemetrexed

MATERIALS & METHODS

The microfluidic addition and removal device, termed the Microformulator, was developed by Prof J. Wikswo and colleagues at VIIBRE, Vanderbilt University (USA). Prior to dosing of cells the device was assembled in a microbiological safety cabinet and the fluidics sterilised with 70% ethanol and flushed with PBS.

Non-small-cell lung cancer lines (NSCLC) H322, H1299, A549, Calu-6 were obtained from Public Health England and cultured in 24-well plates in RPMI supplemented with 10% FCS. Cell viability was assayed using CellTitreGlo (Promega, U.K.). Microscope images were acquired using an Lecia DM IL LED inverted microscope.

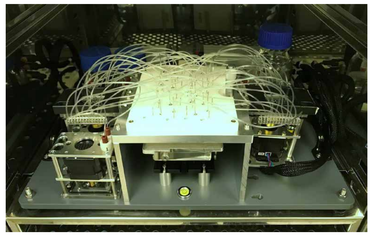

Figure 1 - Microfluidic addition and removal device
The device is installed on a single shelf of a cell culture incubator and it surrounds a single cell culture plate that will be addressed during experimentation.

IN VITRO PK MODELLING

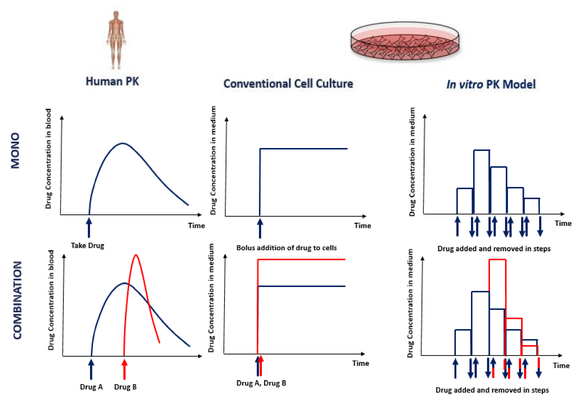

Figure 2 – Modelling of Pharmacokinetics *in vitro*
Schematic representation of how PK varies between *in vivo* and *in vitro* systems and how human PK profiles can be recapitulated *in vivo* using the microfluidic addition and removal device.

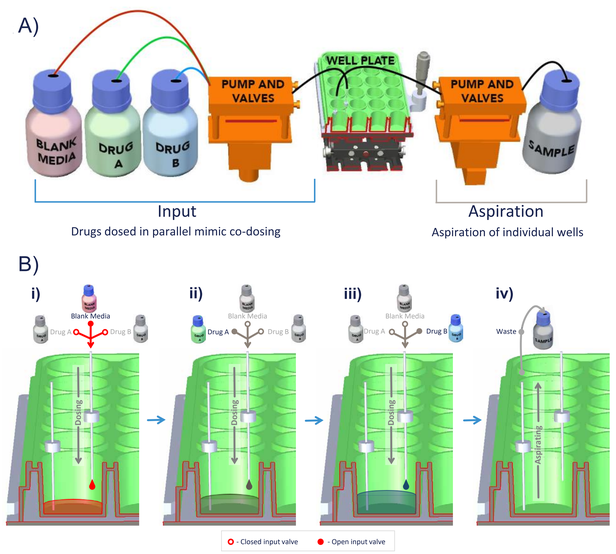

Figure 3 – Microfluidic addition and removal device for PK modelling *in vitro*
A) Schematic representation of microfluidic device operation, where multiple drug inputs are added and removed from individual wells on a cell culture plate. B) Representation of the arrangement of inputs and outputs within a single well of a cell culture plate, i) dosing of blank media, ii) dosing of drug A, iii) dosing of drug B, iv) aspiration of media to waste.

RESULTS

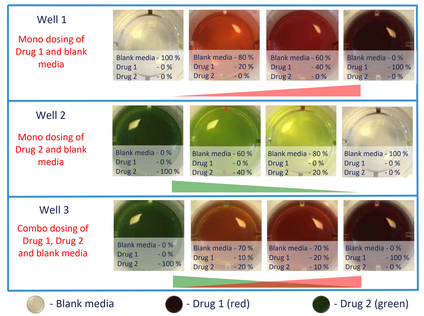

Figure 4 – Combination dosing *in vitro*
Using the microfluidic addition and removal device individual wells are individually addressed and can be dosed with a single compound of interest or a combination of compounds. This dosing is represented here using coloured dye. Drug A = red dye, Drug B = green dye.

Cell Type	Medium Exchange Method	Exchange Frequency (hrs)	% reduction in Growth Rate
Calu-6	Manual	24	28
	Microfluidic Device	24	11
	Microfluidic Device	2	42
H1299	Microfluidic Device	4	46
A549	Microfluidic Device	4	16
H322	Microfluidic Device	8	45

Table 1 – Effect of medium exchange on growth rate
NSCLC cell lines were cultured under the microfluidic device for 3 - 7 days and the was media changed at recurring intervals. The effects on growth rate were assessed and compared to control cells media changed every 72 hours.

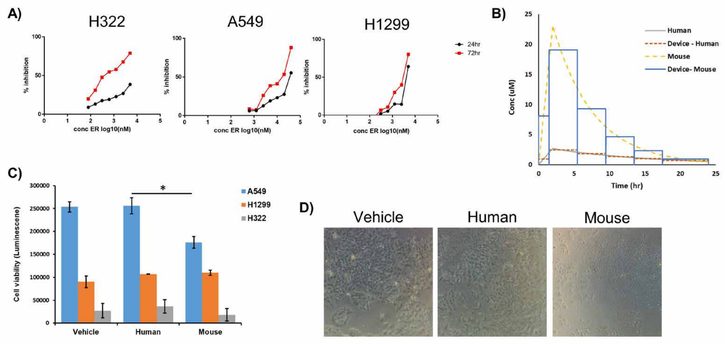

Figure 5 – Erlotinib has varying PK in mouse and humans which influences therapeutic effects
NSCLC cell lines are susceptible to the therapeutic effects of erlotinib. A) Effects on growth inhibition were observed in A549, H322 and H1299 cell lines. B) Representation of the human and mouse PK curves for erlotinib and how these were recapitulated by the microfluidic device. C) NSCLC cell lines were exposed to three different PK profiles of erlotinib with the microfluidic device for 3 days and cell viability was assessed. D) Images for A549 cells following exposure to PK profiles of erlotinib.

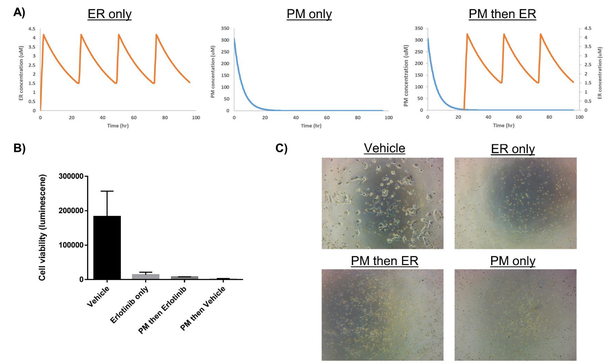

Figure 6 – Erlotinib and pemetrexed co-dosing *in vitro* demonstrates therapeutic effect
A) Schematic representation of the PK profiles and dosing schedule for erlotinib (ER) and pemetrexed (PM), either in mono-dose or co-dose. B) H322 cells were dosed according to the schedules for 4 days before cell viability was assessed. C) Representative images of cells post-dosing.

CONCLUSIONS

The microfluidic device is able to aspirate and then add defined volumes of one or more fluids to individual wells of a multi-well plate. This enables the recapitulation, using sequential steps, of PK like profiles *in vitro*. As a consequence the culture medium within each well is replaced, every few hours, this reduces cell growth, in a cell line dependent manner.

Wells are individually addressable, allowing multiple different compounds, PK profiles, combinations or dosing schedules to be assessed on a single plate. NSCLC lines show varying, time dependent sensitivity to erlotinib. When dosed with PK profiles modelled on mouse (20 mg/kg daily) and human (150 mg daily) inhibition of cell growth was not observed for H1299 cells, but was demonstrated for A549 and H322 cells with the mouse profile.

A combination dosing experiment was developed based on clinical trials protocols for erlotinib and pemetrexed utilising sensitive H322 cells. Pemetrexed or pemetrexed followed by erlotinib were marginally more effective than erlotinib alone, but all conditions showed high levels of inhibition. Further investigations with less sensitive cell lines would be of interest.

ACKNOWLEDGEMENTS AND DISCLOSURES

Thanks to John Wikswo, Ron Reiserer, David Schaffer, Clayton Britt, and Greg Gerken at VIIBRE, Vanderbilt University for support in this work and for the development of the MicroFormulator. Thanks to Anya Lindstrom Battle and Kareene Smith at CN Bio Innovations for support in running experiments. At the time of the study all authors were employees of CN Bio Innovations.

CN Bio Innovations licences from Vanderbilt University intellectual property related to the MicroFormulator.