Investigating the PK/PD/efficacy relationship of PI3K inhibitors *in vitro*, enabled by a microfluidic addition and removal device

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INTRODUCTION

Characterizing the relationship between pharmacokinetics (PK), pharmacodynamics (PD) and efficacy is critical in the discovery and development of new drugs, schedules and combinations. The PK/PD/efficacy relationship has historically been characterized in xenograft models, owing to an absence of viable alternatives. The study of this relationship *in vitro*, has to date been problematic as the generation of time varying concentrations in multi-well plates has not been possible.

Here, we have explored an *in vitro* methodology utilizing a device capable of recapitulating PK-like profiles *in vitro.* Through stepwise addition and removal of medium from the wells of a microtiter cell culture plate the device was able to recapitulate PK-like, time varying concentration profiles of one, or more drugs in individual wells. We used this approach to explore the effects of PI3K pathway inhibitors, on phosphorylated-AKT (p-AKT) levels (PD marker), and viability in a number of cancer lines. The PI3K/mTOR pathway is a central oncogenic pathway deregulated in cancer and p-AKT is a marker of PI3K pathway activity [1]. Two PI3K inhibitors were explored in this study BYL719 [2] and Pi-103 [3] which are known to have varying PK profiles and target coverage of PI3K isoforms.

AIM

Utilise a microfluidic addition and removal platform to i) recapitulate PK profiles of PI3K inhibitors *in vitro*, ii) demonstrate the PK/PD/efficacy relationship of PI3K inhibitors, their effects on p-AKT levels and cell viability.

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 autoclave sterilized, assembled in a microbiological safety cabinet and the fluidics sterilised with 70% ethanol and flushed with PBS between experiments.

Cancer lines T47D (breast cancer), Colo205 (colon cancer) and A549 (non-small cell lung cancer) 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, UK) and Alamar Blue (Promega, UK). Microscope images were acquired using an Lecia DM IL LED inverted microscope. Phospho-AKT1 (S473) levels were measured by ELISA kit (R&D Systems).

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

IN VITRO PK MODELLING

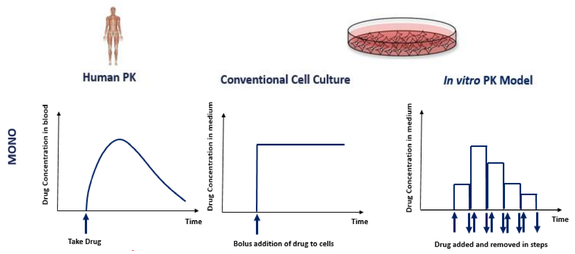

Figure 1 – Modelling of Pharmacokinetics *in vitro*
Schematic representation of how PK varies between *in vivo* and *in vitro* systems and how human PK profiles can be recapitulated *in vivo* using the microfluidic addition and removal device.

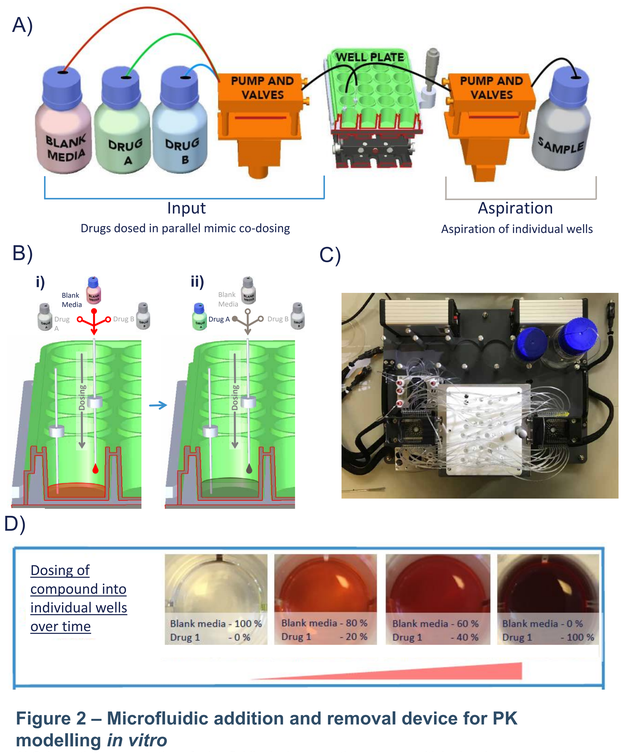

Figure 2 – Microfluidic addition and removal device for PK modelling *in vitro*
A) Schematic representation of microfluidic device operation, where multiple drug inputs are added and removed from individual wells on a cell culture plate. B) Representation of the arrangement of inputs and outputs within a single well of a cell culture plate, i) dosing of blank media, ii) dosing of drug. C) Top down view of microfluidic addition and removal device on benchtop. D) Representative images of compound dosing into wells over time using device.

RESULTS

Cell line	BYL719 GI_{50} (μM)	BYL719 IC_{50} (μM)	PI-103 GI_{50} (μM)	PI-103 IC_{50} (μM)
A549 (lung)	12.5	191.7	0.6	857.5
Colo205 (Colon)	8.1	N/A	12.32	N/A
T47D (breast)	6.7	77.6	0.8	65.8

Table 1 – Effect of PI3K inhibitors on tumour growth and phospho-AKT levels
Various cancer cell lines were cultured for 72 hours in the presence of PI3K inhibitors BYL719 or PI-103 and cell viability was assessed by Cell Titre Glo to determine GI_{50} concentrations. Equivalent cells were also dosed for 2 hours and the intracellular levels of phospho-AKT assessed by ELISA to determine IC_{50} concentrations. N/A = cell line not sensitive.

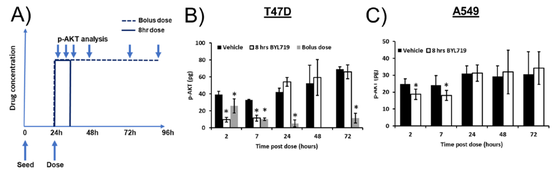

Figure 3 – Short term exposure of PI3K inhibitor causes the lose and then restoration of cellular phospho-AKT levels
T47D and A549 cell lines are susceptible to the therapeutic effects of PI3K inhibitor BYL719, but cellular p-AKT levels recover when the compound is removed. A) Dosing schedule – cells dosed for 8 hours and then washed and left in culture for 64 more hours or bolus dosed (72 hrs with compound). Arrows indicate timepoints for analysis. B) BYL719 dosed T47D cells, p-AKT levels were measured at various time points after inhibitor dosing. C) BYL719 dosed A549 cells, p-AKT levels were measured at various times after dosing. Data shown are Mean ± SD. * = P < 0.05.

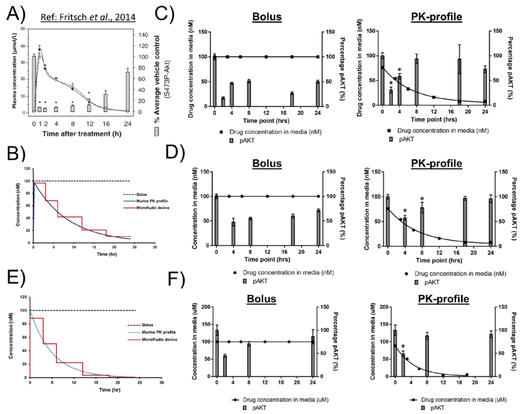

Figure 4 – Mimicking *in vivo* BYL719 and PI-103 exposure *in vitro* causes time/dose dependent loss of cellular p-AKT
The murine PK for BYL719 can be recapitulated by the microfluidic device and used to dose cells *in vitro*. A) The PK/PD relationship in mice for BYL719 and p-AKT in Rat1-myr-p110α tumors as determined by Fritsch *et al.* [2]. B) The *in vitro* PK-like profile of BYL719, compared to bolus dosing. C) Effects on p-AKT in T47D cells dosed with either a bolus dose of BYL719 or a PK-like profile. D) Effects on p-AKT in A549 cells dosed with either a bolus dose of BYL719 or a PK-like profile. E) The *in vitro* PK-like profile of PI-103, compared to bolus dosing. F) Effects on p-AKT in T47D cells dosed with either a bolus dose of PI-103 or a PK-like profile. Data shown are Mean ± SD. * = P < 0.05

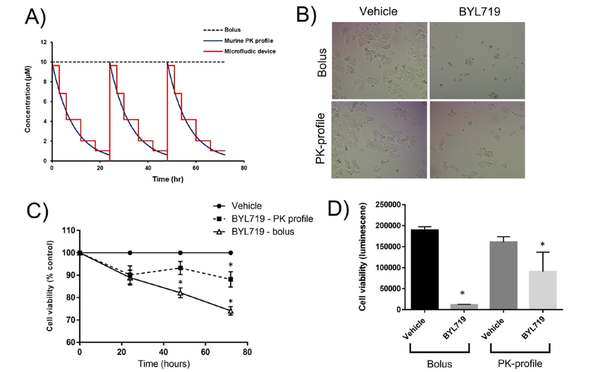

Figure 5 – *In vitro* PK-like exposure profile of BYL719 induce varying efficacy responses compared to bolus doses in T47D cells
T47D cells were dosed with A) the *in vitro* PK-like profile of BYL719, or bolus dosing over a 72 hour experiment. B) Representative images of T47D cells post dosing. C) Growth curves of T47D cells exposed to either vehicle, bolus BYL719 or the *in vitro* PK profile of BYL719, with level of cell growth determined by Alamar Blue viability assay or D) Cell titre Glo. Data shown are Mean ± SD. * = P < 0.05.

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*.

Dosing of PI3K inhibitors onto cell lines for distinct periods of time demonstrated a dose-dependent decrease in phospho-AKT, which recovered when the compound was removed. The PK profile for BYL719 (50 mg/kg QD) was modelled and a dose and time dependent decrease in p-AKT was observed in both T47D and A549 cells, mirroring *in vivo* observations [2]. The PK profile of PI-103 was also modelled [3] which caused a time dependent decrease in p-AKT, however similar observations were observed with bolus dosing suggesting the compound is unstable in the culture conditions.

When subjected to multiple PK exposure profiles *in vitro* T47D cells demonstrated an altered efficacy response (growth arrest) compared to standard bolus dosing. This data demonstrates how the microfluidic device enables for the first time PK/PD/efficacy relationship studies to be performed *in vitro*.

ACKNOWLEDGEMENTS AND DISCLOSURES

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