IN VITRO PK MODELLING

RESULTS

CONCLUSIONS

ACKNOWLEDGEMENTS AND DISCLOSURES

REFERENCES

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 vitro using the microfluidic addition and removal device.

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. Whissoo and colleagues at VIBiore, 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 assessed using CellTiterGlo (Promega, UK) and Alamar Blue (Promega, UK). Microscope images were acquired using an Leica DM IL LED inverted microscope. Phospho-AKT1 (S473) levels were measured by ELISA kit (R&D Systems).

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.

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

Dharminder Singh, Paul Golby, Alysha Bray, Tomasz Kostrezewski, David Hughes
CN Bio Innovations Limited, BioPark Hertfordshire, Welwyn Garden City, UK, AL7 3AX. Correspondence: david.hughes@cn-bio.com

INTRODUCTION

Characterising 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 characterised 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 plate 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 oncopathic 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.

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 on a cell culture plate. I) dosing of blank media. II) dosing of drug. C) Top down view of microfluidic addition and removal device on bench. D) Representative images of compound dosing into wells over time using device.

Figure 3 – Short term exposure of PI3K Inhibitor causes the lose and restoration of cellular phospho-AKT levels
T47D and A549 cell lines were 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 9 hours and then washed and left in culture for 54 more hours or bolus dosed (72 hrs with compound). Arrow indicate timepoints for analysis. B) BYL719 treated T47D cell p-AKT levels were measured at various time points after inhibitor dosing. C) BYL719 treated A549 cells, p-AKT levels were measured at various time points 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 curve fit for BYL719 can be recapitulated on the microfluidic device and used to dose cells in vitro. A) The PK/PD relationship in mouse for BYL719 and PI-103 is linear and fits to a 1/D model as determined by Paluch et al [2]. B) The in vitro PK/PD profiles of a bolus dose to BYL719 compared to bolus dosing. C) Effects of p-AKT on PI3K cells dosed with a bolus dose of BYL79 or PI-103 profile. D) Time in vitro PI3K profile of PI-103, compared to bolus dosing. E) Effects on p-AKT in T47D cells dosed with either a bolus dose of PI-103 or a PI-103 profile. Data shown are Mean ± SD. ** P<0.05

Table 1 – Effect of PI3K inhibitors on tumour growth and phospho-AKT levels

<table>
<thead>
<tr>
<th>Cell line</th>
<th>BYL719 IC50 (µM)</th>
<th>BYL719 GI50 (µM)</th>
<th>PI-103 IC50 (µM)</th>
<th>PI-103 GI50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549 (lines)</td>
<td>12.5</td>
<td>191.7</td>
<td>0.6</td>
<td>857.5</td>
</tr>
<tr>
<td>Colo205 (Col)</td>
<td>8.1</td>
<td>N/A</td>
<td>12.3</td>
<td>N/A</td>
</tr>
<tr>
<td>T47D ( lines)</td>
<td>6.7</td>
<td>77.6</td>
<td>0.8</td>
<td>65.8</td>
</tr>
</tbody>
</table>

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

No sources of funding, grants, or support were needed for this work.

REFERENCES

1. Paluch, F.: Pharmacokinetic & dynamic PK/PD relationships in solid tumors. From Laboratory to the clinic. AACR Conference Coverage, 2019