

# A semi-mechanistic PK/PD model of vemurafenib resistance and its rescue by LY2835219, a cyclin-dependent kinase 4/6 inhibitor, in mice bearing human melanoma xenograft tumours

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## ABSTRACT

**Background:** Although vemurafenib demonstrates excellent clinical efficacy in the first-line treatment of BRAF V600E-mutated metastatic melanoma (1), resistance ultimately develops and patients relapse (2). Experimental evidence in resistant melanoma cells indicates that MAPK pathway reactivation is a primary mechanism for resistance (3,4); however recent studies indicate that the acquisition of resistance may also be associated with an activation of the CDK4/6 pathway through upregulation of cyclin D1 (5,6). Consistent with these findings, CDK4/6 inhibition by LY2835219 overcomes resistance and produces tumour growth inhibition in vemurafenib-resistant A375 melanoma xenografts (6). The objective of this study is to develop an integrated pharmacokinetic (PK)/pharmacodynamic (PD) model to characterize resistance to vemurafenib and its rescue by LY2835219 in A375 tumour xenografts.

**Methods:** The semi-mechanistic PK/PD model previously developed to describe cell cycle inhibition by LY2835219 (7) was extended to include vemurafenib-mediated BRAF inhibition on the MAPK pathway. Tumour shrinkage induced by vemurafenib was described by inhibition of pERK (major route) and pHH3 (minor route). A modulator compartment driving time-dependent up-regulation of the MAPK pathway was incorporated to account for emerging vemurafenib resistance and increasing sensitivity to total Rb. Finally, rescue by LY2835219 was associated with LY2835219-mediated inhibition of total Rb.

**Results:** Vemurafenib-mediated tumour shrinkage was adequately described by the extended biomarker model. Inhibition of pERK was confirmed to be the primary contributor to tumour shrinkage, and a minor contribution of cyclin D1-mediated cell cycle arrest was identified. Resistance to vemurafenib was successfully accounted for by time-dependent over-expression of pMEK, pERK and cyclin D1. More importantly, inclusion of cyclin D1-mediated sensitivity to total Rb allowed LY2835219-mediated rescue of tumour shrinkage in resistant cells to be successfully characterised.

**Conclusions:** The PK/PD model successfully described the effect of LY2835219 in vemurafenib-resistant A375 melanoma xenografts. Vemurafenib anti-tumour effect and tumour resistance, followed by LY2835219-mediated rescue were described by an integrated semi-mechanistic PK/PD model. These results support the hypothesis that vemurafenib-resistant melanoma cells rely on total Rb levels for survival and support further exploration of the combination of LY2835219 and B-RAF inhibitors in melanoma.

## BACKGROUND

### Resistance to vemurafenib

Resistance to vemurafenib is associated with MAPK pathway reactivation (3-6):

- Although vemurafenib still inhibits the MAPK pathway, resistance-induced upregulation results in elevation of pERK above normal baseline levels at maximum inhibition (8)
- Cyclin D1 up-regulation (6)

### Rescue by LY2835219

The cyclin D1 up-regulation observed in vemurafenib-resistant melanoma cells appears to render them dependent on total Rb for survival (6)

Inhibition of CDK4/6 by LY2835219 overcomes resistance and induces apoptosis, which is thought to be mediated by LY2835219-mediated inhibition of total Rb (6)

## STUDY OBJECTIVE

To develop a semi-mechanistic PK/PD model to quantitatively investigate resistance to vemurafenib and its rescue by LY2835219 in A375 xenograft tumours

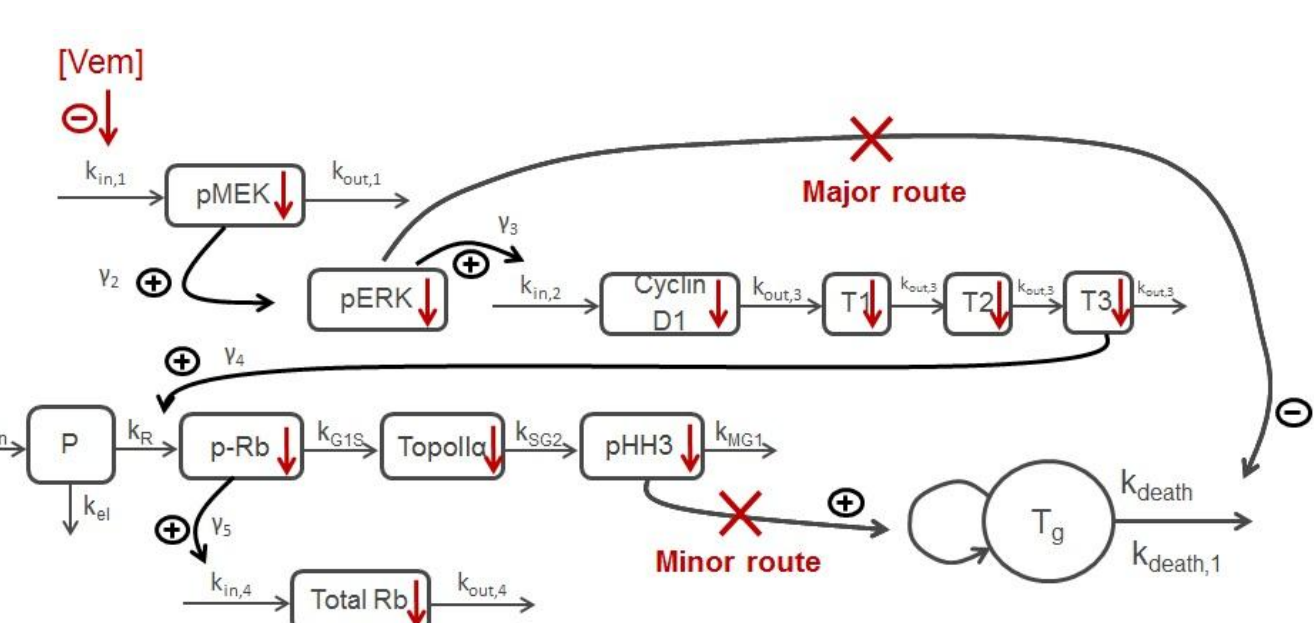
## METHODS

### Effect of vemurafenib

The cell cycle PK/PD model previously developed for LY2835219 (7) was extended to include the MAPK pathway:

- The initial cell cycle phase distribution was re-calibrated to match that of A375 cells (7)
- The MAPK pathway inhibited by vemurafenib (9) was added to the model and connected to the cell cycle by means of cyclin D1 (10)
- The effect of pRb decrease on the levels of total Rb (11) was incorporated to the model
- The effect on tumour growth is mediated by inhibition of pERK (causing apoptosis) and pHH3 (causing cell cycle arrest)

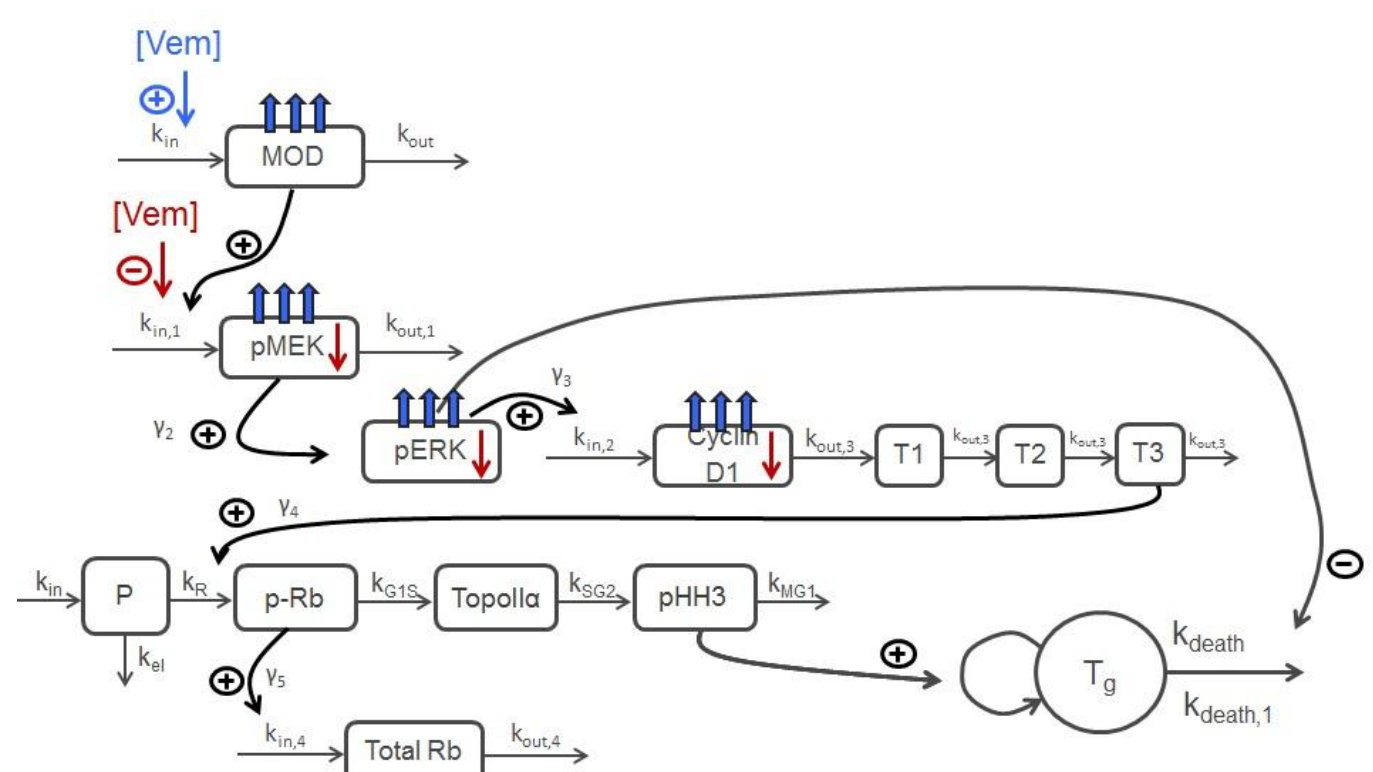
**Figure 1. Schematic representation of the model describing the effect of vemurafenib on A375 tumour xenografts**



### Resistance to vemurafenib

Resistance to vemurafenib was described in the model by means of a vemurafenib-activated modulator compartment driving time-dependent up-regulation of the MAPK pathway

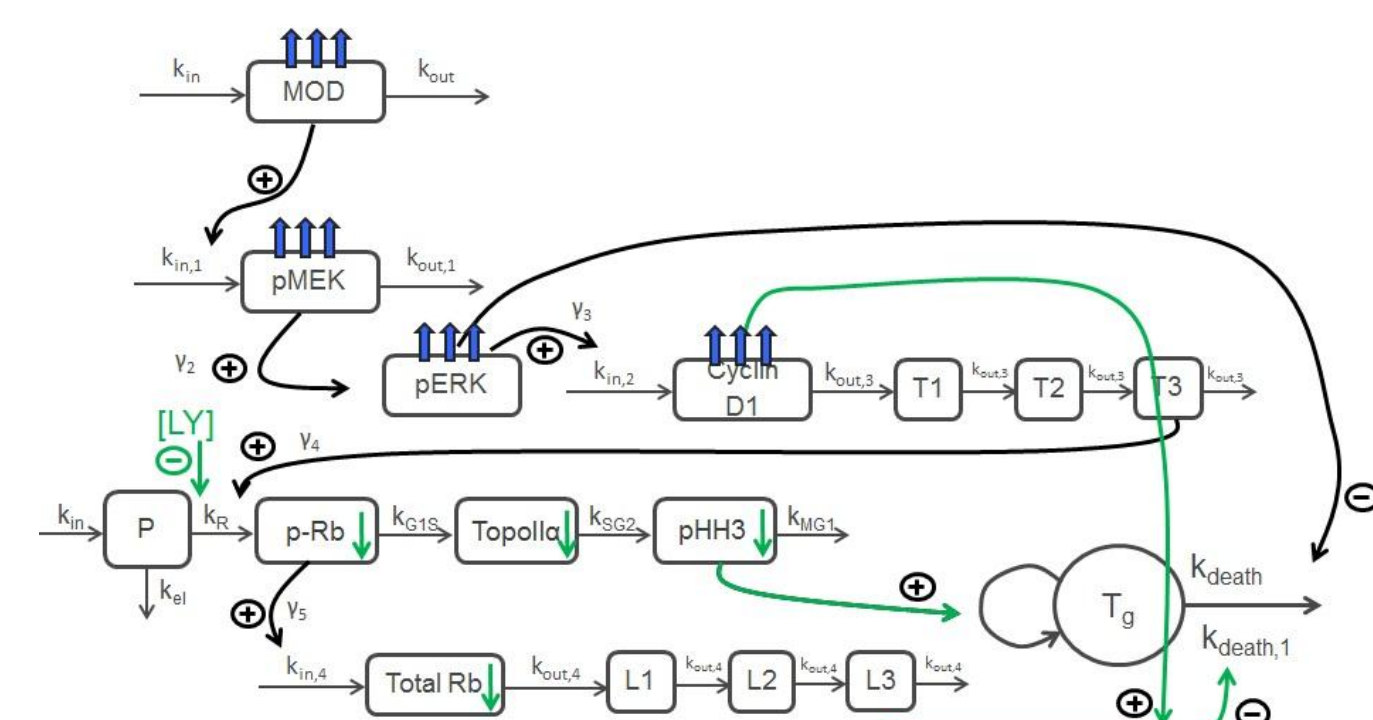
**Figure 2. Schematic representation of the model with MAPK pathway up-regulation as a result of acquired resistance to vemurafenib**



### Rescue by LY2835219

The rescue by LY2835219 was incorporated by LY2835219-mediated pRb inhibition and the subsequent down regulation of total Rb in the cell. If cyclin D1 is overexpressed, inhibition of total Rb causes tumour shrinkage

**Figure 3. Schematic representation of the model where resistance to vemurafenib is overcome by LY2835219-mediated inhibition of total Rb**



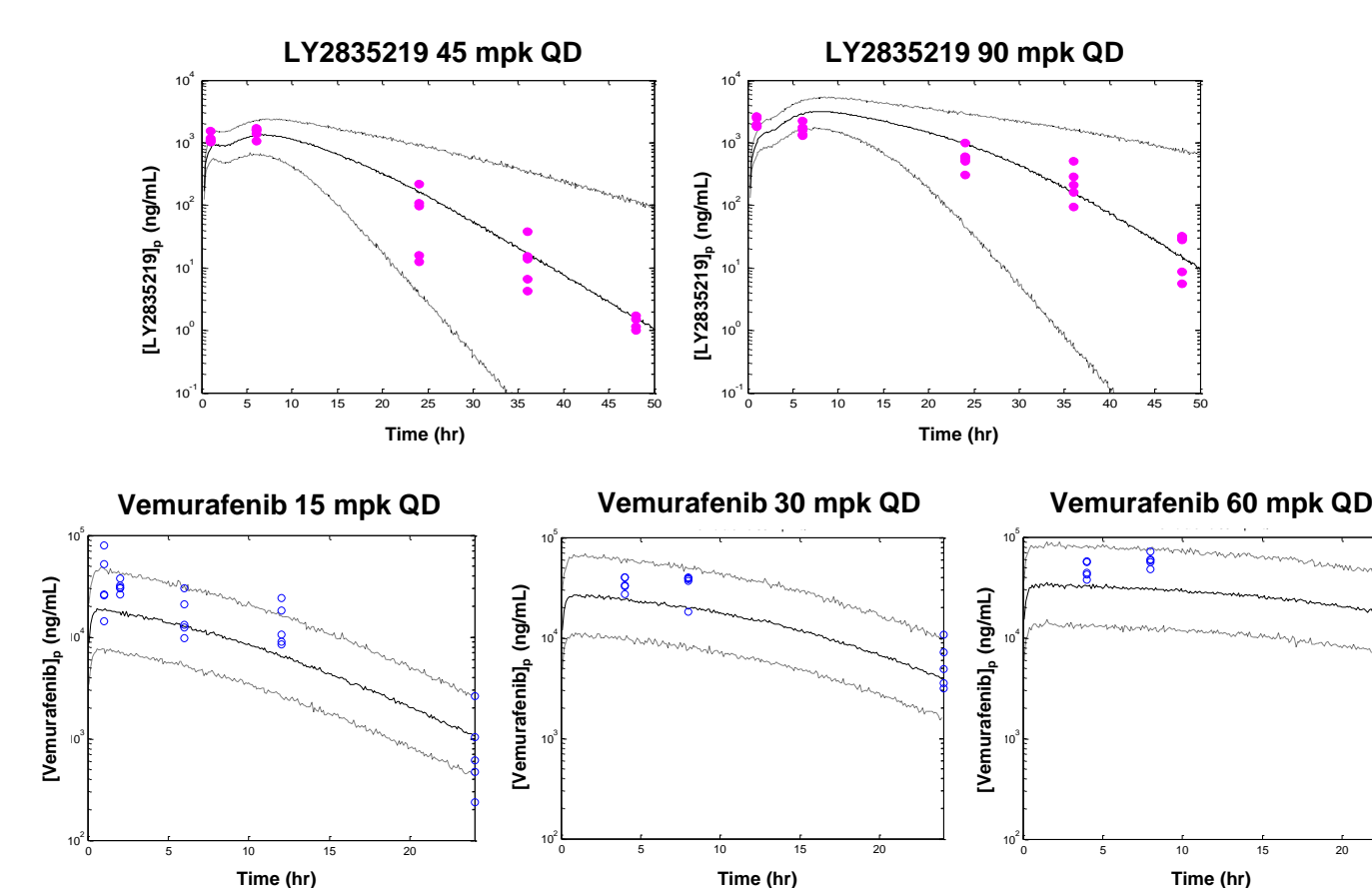
## RESULTS

### LY2835219 and vemurafenib pharmacokinetics

The PK of LY2835219 was adequately predicted by the PK model previously developed in mouse (7).

The PK of vemurafenib was best described by a one-compartment model with linear disposition and saturable absorption

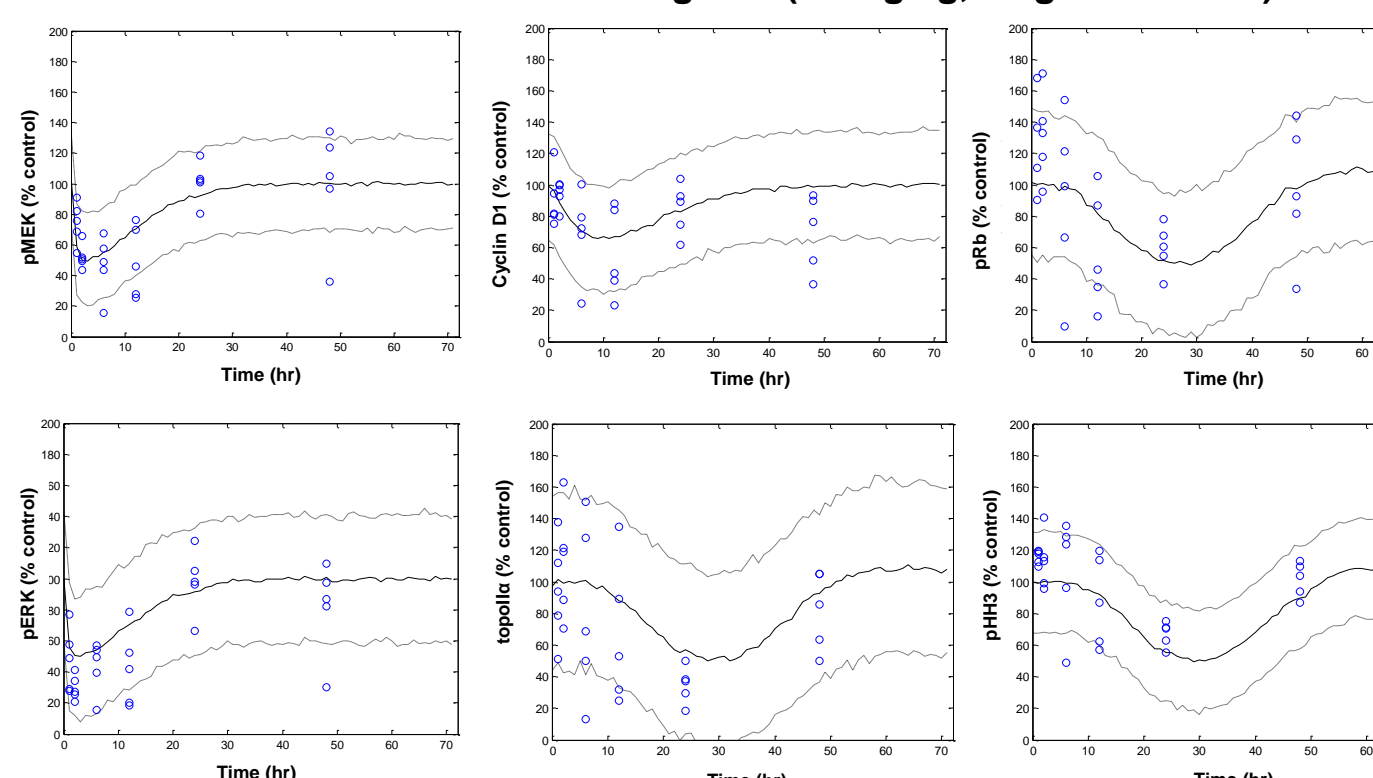
**Figure 4. VPC of the PK model for LY2835219 and vemurafenib**



### Effect of vemurafenib on A375 melanoma xenografts

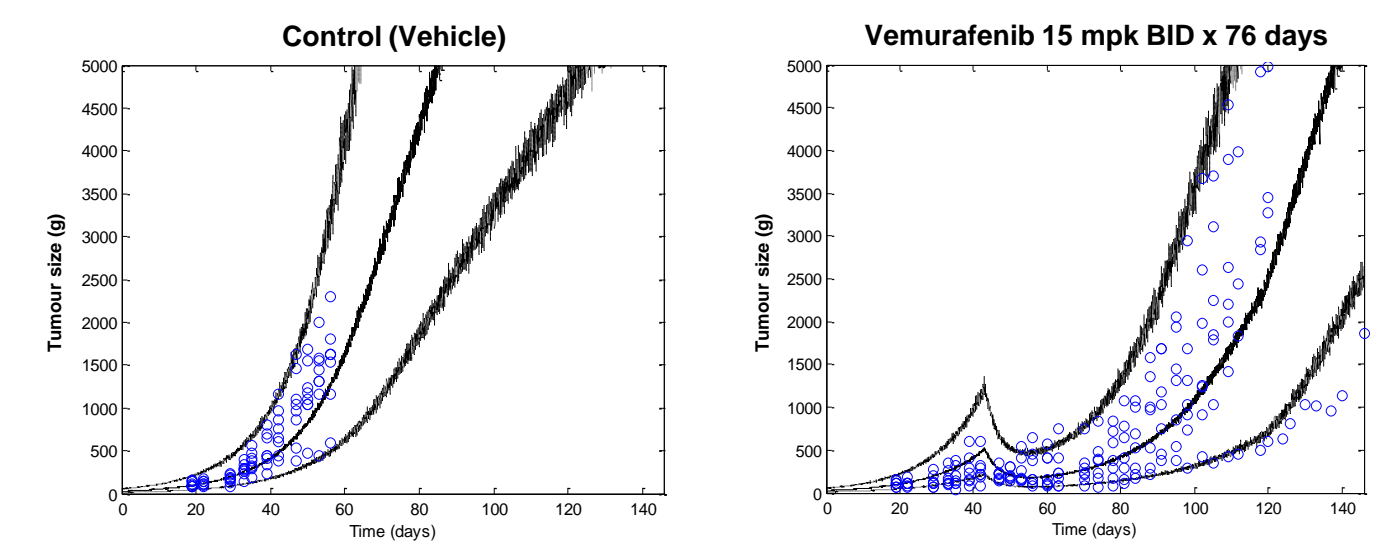
The biomarker model (Fig. 1) was able to describe the effect of vemurafenib on the MAPK pathway. The cyclin D1-mediated transient inhibition of the cell cycle was also adequately captured (Fig. 5)

**Figure 5. VPC – inhibition of the MAPK pathway and cell cycle by vemurafenib in A375 xenografts (15 mg/kg, single oral dose)**



The model was able to capture the anti-tumour activity of vemurafenib (Fig. 6). The addition of a modulator upregulating the MAPK pathway in a vemurafenib-dependent manner (Fig. 2) proved effective at capturing developing resistance.

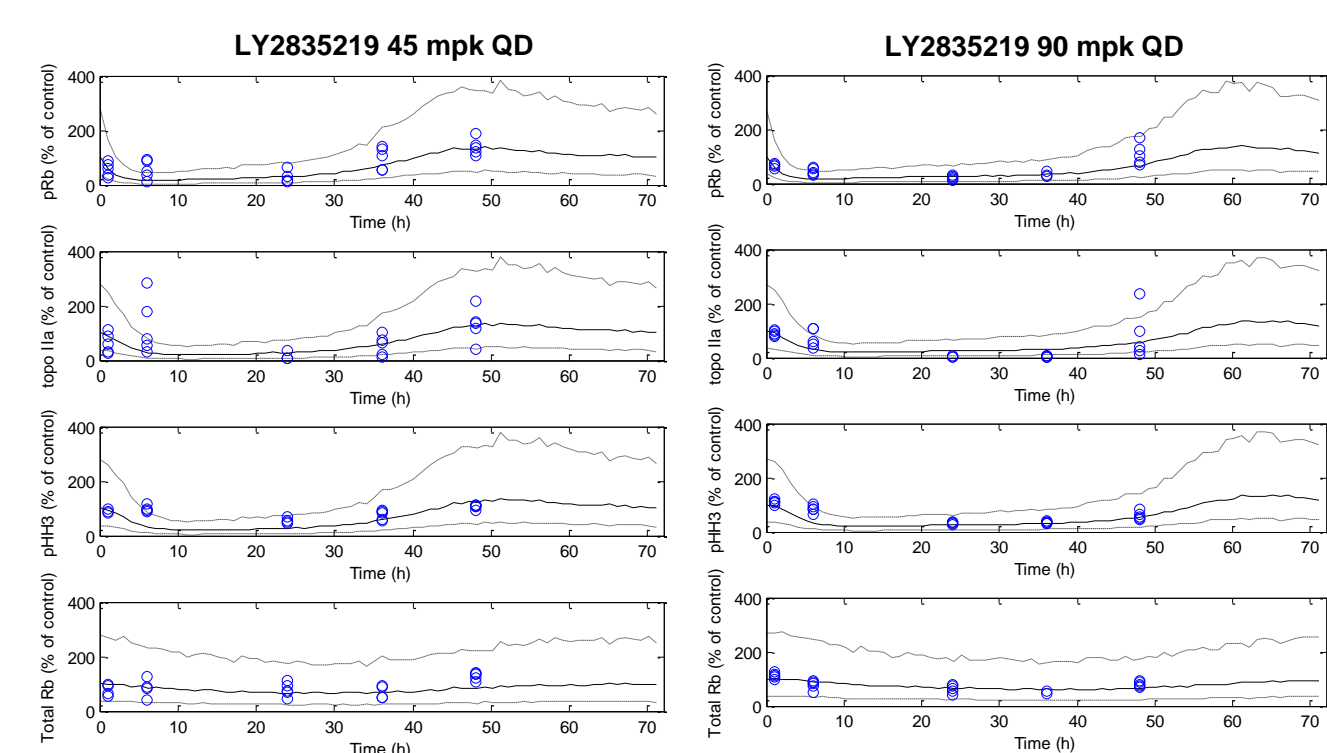
**Figure 6. VPC – time course of tumour growth in A375 xenografts treated with vehicle (left panel) or with vemurafenib**



### Effect of LY2835219 on vemurafenib-resistant A375 xenografts

Prior to testing the effect of LY2835219 on tumour growth, the model previously developed for LY2835219-mediated cell cycle inhibition was validated externally using data from A375 cells (Fig. 7)

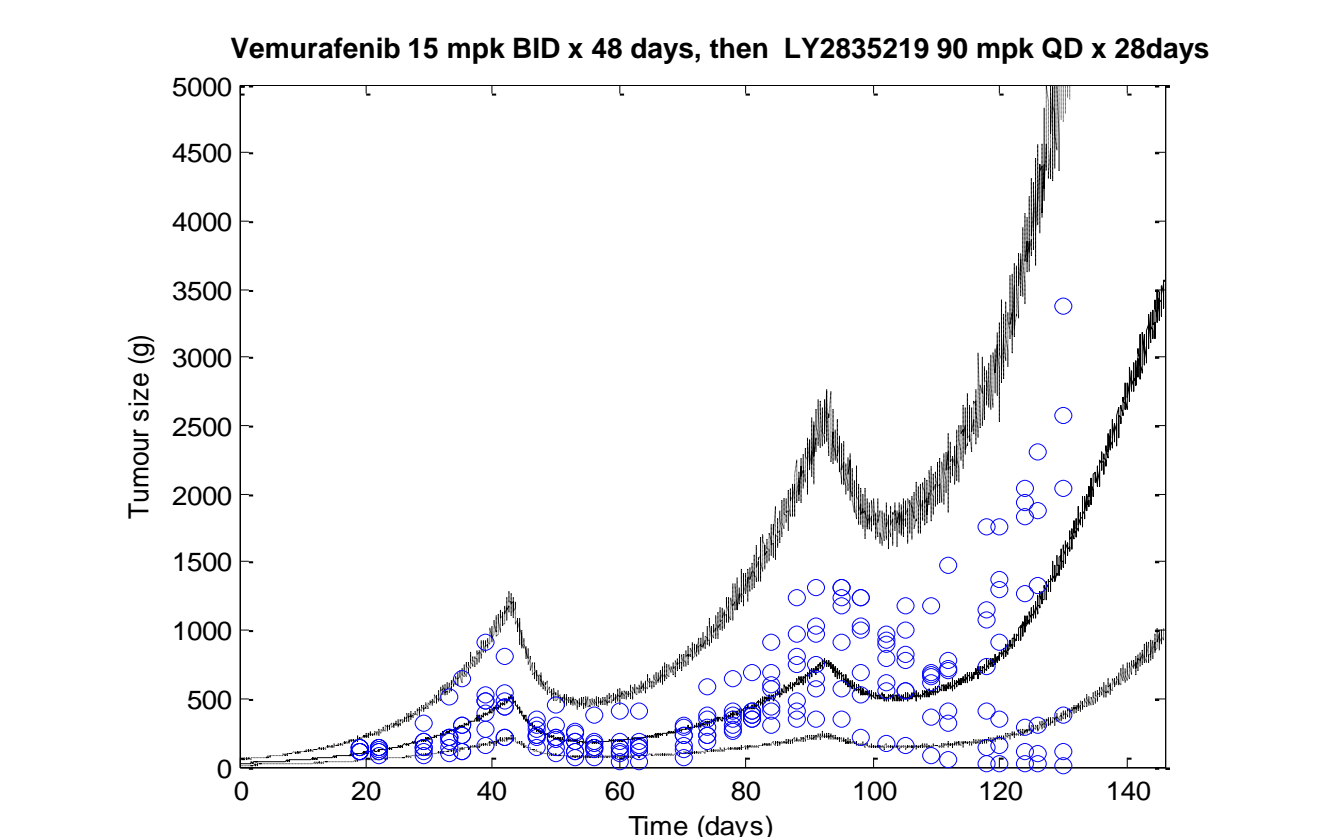
**Figure 7. VPC – time course of cell cycle inhibition by LY2835219 in A375 xenografts (single dose)**



The integrated model described in figure 4 could account for the entire sequence of events including:

1. Response to vemurafenib
2. Resistance to vemurafenib
3. Rescue of vemurafenib-resistant A375 xenografts by LY2835219

**Figure 8. VPC – time course of tumour growth in A375 xenografts treated with vemurafenib followed by LY2835219**



## CONCLUSIONS

- A semi-mechanistic PK/PD model has been established that describes vemurafenib anti-tumour activity followed by resistance in A375 xenograft tumours
- The semi-mechanistic nature of the model allowed to explore the following aspects of resistance to vemurafenib and LY2835219-mediated rescue:
  - Resistance to vemurafenib could be accounted for by primarily upregulating the expression of pERK, pMEK, cyclin D1
  - Reliance on total Rb levels in the context of cyclin D1 upregulation successfully captured LY2835219-mediated rescue by means of its activity on the Rb pathway

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