

# Combination of Oncolytic Reovirus and Rapamycin in a B16.F10 Mouse Melanoma Model



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## Introduction

•Reovirus type 3 Dearing (T3D) is an oncolytic virus which has demonstrated anticancer activity *in vitro*, *in vivo* and after systemic delivery in humans. It is currently undergoing evaluation in a number of clinical trials.

• Though it has shown activity as a single agent, these trials are focusing on its combination with chemotherapy, and radiotherapy, to try and improve its efficacy.

•mTOR is an important downstream kinase in the PI3K/Akt signalling pathway. Dysregulation of PI3K signalling occurs in many common cancers.

•mTOR inhibitors have shown promise as anticancer agents in the clinical setting, and synergy with other oncolytic viruses has been demonstrated in animal models. Oral mTOR inhibitors are now available in clinical practice.

•In this study, we examined the *in vitro* and *in vivo* oncolytic activity of reovirus T3D against the mouse melanoma cell line B16.F10 in combination with the mTOR inhibitor rapamycin.

## 1. Synergistic Anti-tumour activity between Reovirus and Rapamycin is Sequence-Dependent

Using a constant ratio combination design and the combination index method based on the Chou and Talalay median-effect principle (1), the effect of reovirus combined with rapamycin on B16.F10 cells was assessed.

Cells ( $5 \times 10^3$ /well) were seeded in 96 well plates and allowed to adhere overnight. Culture medium was replaced with doubling dilutions of rapamycin and/or reovirus, corresponding to 2, 1, 0.5 and 0.25 x the previously determined ED50, diluted in fresh culture medium and incubation continued for 48h. At this time, medium was removed and percentage cell survival compared to untreated cells was determined using the MTS assay. Data were analysed using the CalcuSyn programme.

The effect of sequencing was assessed by adding the rapamycin 24 hours before or after the reovirus. Of note, at 24h little if any cell death was seen with reovirus. The interaction was antagonistic (combination index value {CIV} of more than one) if the rapamycin preceded or was given concomitantly with reovirus (figures 1a and 1b respectively). A synergistic interaction (CIV of less than one) was observed between reovirus and rapamycin only when the rapamycin was given after the reovirus (Figure 1c).

Figure 1a

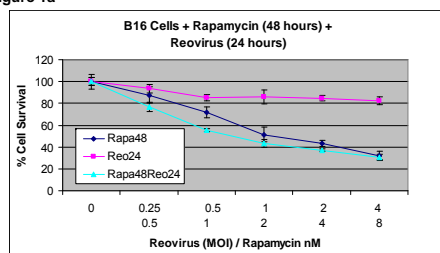


Figure 1b

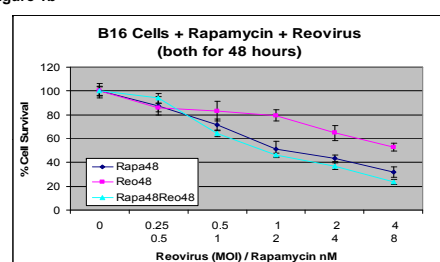


Figure 1c

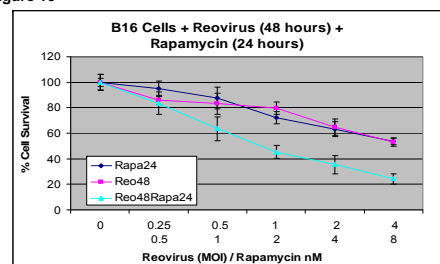


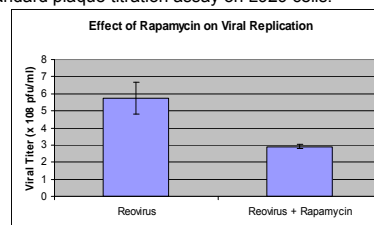
Table 1. Combination Indices

	ED50	ED75	ED90
Rapamycin→ Reovirus	1.29±0.10	1.86±0.08	2.65±0.3
Rapamycin/ Reovirus	1.41±0.12	1.74±0.20	2.58±0.77
Reovirus→ Rapamycin	0.51±0.02	0.46±0.07	0.48±0.1

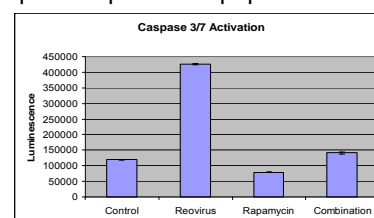
Data represents CIV ± SEM at the effective dose indicated

## 2. Concomitant rapamycin inhibits viral replication *in vitro*

The effect of concomitant rapamycin on reovirus replication was assessed by viral plaque assay. 6-well plates seeded with  $5 \times 10^5$  cells were exposed to reovirus MOI 10 with or without rapamycin 8nM for 48 hours. Viral titers were quantified 72 hours after infection using a standard plaque titration assay on L929 cells.



## 3. Effect of rapamycin on reovirus caspase dependent apoptotic cell death



Following overnight adherence in 96-well plates, cells were exposed to reovirus (MOI 2) and/or rapamycin (4nM) diluted in fresh culture medium and incubated for 48h. Caspase 3/7 activation was quantified using Caspase-Glo luminescent assay (Promega). When given concomitantly rapamycin appears to decrease the caspase activation seen with reovirus alone.

## Conclusions

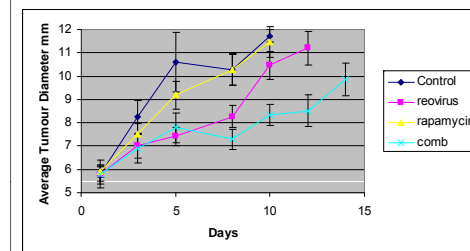
Synergy between rapamycin and reovirus *in vitro* is sequence dependent, and only seen when rapamycin is administered after reovirus.

•Concomitant administration of rapamycin and reovirus decreases apoptotic cell death and viral replication. We are currently testing the effect of sequencing on viral replication and apoptosis.

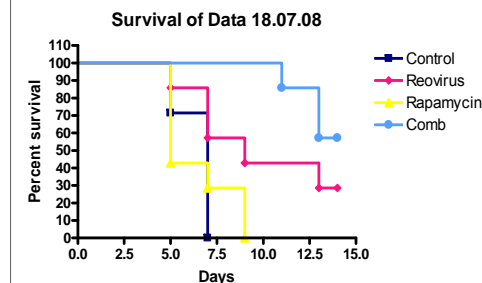
•Unlike the *in vitro* observations, the combination of rapamycin and reovirus is synergistic *in vivo* and we are assessing the effect of sequencing to enhance this effect.

## 4. Combined reovirus and rapamycin therapy reduces the growth of subcutaneously implanted tumours and prolongs median survival time

In the *in vivo* setting, B16.F10 tumours were seeded subcutaneously in C57Bl/6 mice and treated with intratumoural reovirus T3D  $5 \times 10^8$  TCID50 on day 1 and 4, and intraperitoneal rapamycin 5mg/kg on day 1, 4, 8 and 12 either alone or in combination, or with control treatment (intratumoural PBS, intraperitoneal PBS).



The diameter of each tumour was measured and an average calculated for each group. Combined reovirus T3D/ rapamycin treatment resulted in markedly reduced tumour growth compared to single agent treatments or control treatment.



Survival was plotted as a Kaplan-Meier curve. Median survival time for control treated mice was 7 days. There was no improvement in median survival with rapamycin alone. Reovirus alone prolonged median survival time 9 days. Combined therapy increased median survival time to >15 days (Logrank test  $p = 0.0216$ ).

References: 1) Chou, T.-C and Talalay, P. Analysis of combined drug effects: A new look at a very old problem. Trends Pharmacol. Sci. 4:450-454, 1983