Chapter 7

Conclusions

The main results of this project are outlined at the end of chapters 3 – 6. In this chapter, I will outline the overall conclusions derived from this project with particular focus on mathematical modelling and systems biology and discuss possible future work.

7.1 Conclusions

The aim of this project was the development of a mathematical model of cell growth based on the movement of cells through stages of the cell cycle and alternatively into states of senescence and/or cell death. The models would then be used to probe the mechanism of action of a new anti-tumour agent, RHPS4 and determine where in the cell cycle this agent is likely to act. An anti-tumour agent with a known mechanism of action was also investigated to test the model and determine that this method of analysis is able to distinguish between two molecules. The data we can derive easily in the laboratory provides information on the effects of anti-tumour agents on the cell cycle, senescence levels and population doublings. Flow cytometry incorporating PI allows information on the proportion of cells in the cell cycle phase's pre-G_1, G_1/G_0, S and G_2/M to be gained, though does not distinguish between cells in G_1 and G_0 or cells in G_2 and M (Fried *et al*, 1976). The incubation of the cells with anti-tumour agents in a range of concentrations over time allows conclusions regarding the effects of concentration and time to be generated on the phases of the cell cycle. Data on population doublings allows information on the effects on the growth rate to be determined, whilst the SA-β-gal staining method allows information on the effects on the proportion of senescent cells to be generated. Not easily derived in the laboratory are the effects of anti-tumour agents on the kinetics of the cell cycle and

how these are affected by concentration and time. For example, whilst the cell cycle data may draw the conclusion of an increase in the proportion of cells in G_1/G_0, is this due to a reduction in the rate at which cells are leaving these phases to enter S phase or is there an overall increase in the rate of cells entering from G_2/M. It is also possible that there is a reduction in the transition of cells from G_0 to G_1. The mathematical model aims to resolve this and derive a system of ODEs to allow the rate of the movement of cells between compartments of the cell cycle under normal conditions and in the presence of anti-tumour agents to be determined. In doing this we are utilizing the data available to generate further conclusions and give indications of the mechanism of action of anti-tumour agents and focus further research.

7.2 Mathematical Modelling: Requirement of Parameters

A model is a system of mathematical equations, those being algebraic, differential, partial differential or integral that allows all known experimental data to be incorporated and must involve the best possible understanding of the biological phenomenon in question and the possibility of functioning (Cherruault, 1986). Models are created with a particular purpose in mind and often this is to make predictions and successful ones provide a relationship between relevant variables from which the necessary answers are obtained. The predictions of truly successful mathematical models can be verified with the available data (Edwards & Hamson, 1989). Although not a traditional experimental 'method', mathematical modelling provides a powerful approach to investigating complex biological systems, such as the cell cycle (Sible & Tyson, 2007). There are different types of mathematical models and each uses mathematical language to describe a system and explain the dynamics of that system: linear or non-linear, static or dynamic, deterministic or probabilistic and lumped or distributed parameters (Gershenfeld, 1999; Edwards & Hamson, 1989).

Parameterisation of our mathematical model required robust data regarding the cell cycle, senescence and population doublings from a well-characterised cell line. Chapter 3 describes the approach to the selection of this cell line from a series of

colorectal cell lines, those being HCT116, HT-29, KM12 and HCC-2998. Colorectal cell lines were used to keep in line with an EPSRC funded project in escience (an integrative biology project). The aim of this project is to build a multiscale computational model of colorectal cancer. Each of the cell lines were put through a series of biological assays including MTT assays for cell viability studies, flow cytometry incorporating PI for cell cycle studies and short-term population doublings. The aim of these initial studies was to determine a 'best-behaved' cell line which would be used to generate more detailed data of the anti-tumour agents and be applied to the mathematical models to give insight into their mechanism of action. The desired characteristics were:

1. Good growth characteristics – the selected cell line should grow to 60-70% confluence with relative ease.
2. Robustness – in this context, robust means the relative insensitivity of the cell line to alteration of their internal parameters and the ability to adapt to changes in their environment (Sabău, 2005). We wanted to observe the effects of anti-tumour agents on the selected cell line prior to cell death.
3. Reliability and tractability - the chosen cell line would be reliable in a range of biological assays. Systems biology approaches rely heavily on information available in public databases which are often incomplete or not standardized and so the data would have to be reproducible and of a high quality (Aderem, 2005). High quality data is needed if we are going to take the systems biology approach.

7.3.1 Initial Studies of the Colorectal Cell Lines

The initial studies were to determine the effects of a range of concentrations of RHPS4 and revealed that the HCT116 and HT-29 cell lines were the most reliable in terms of the reproducibility and they were also the most robust as they showed cell cycle perturbations without relatively low proportions of cell death. MTT assays revealed that the KM12 cell line was the most sensitive to RHPS4. Regardless of this property, KM12 cells had an undesirable morphology as they

tended to grow in 'clumps' as opposed to a flat monolayer which was observed in the other cell lines. In addition to MTT assays, analysis of the short-term effects of RHPS4 on each of the cell lines was also carried out. The cells were incubated in RHPS4 at concentrations of 100 nM, 1 μM and 10 μM and analysed between 1 - 24 hours. These results revealed that very little occurred up to 24 hours with regards to the cell cycle for each cell lines at the lower concentrations of RHPS4. The HCC-2998 cell line was the most unreliable and it was evident that they took the longest time to establish within the cell culture flasks. In addition to this, the HCC-2998 cell line had the longest doubling time of 32 hours, whilst the shortest doubling time was the HCT116 cell line of 17 hours.

It was evident from MTT assays there was a reduction in cell viability with no cell cycle perturbations at early time points. As we wanted to observed cell cycle perturbations, the longer-term cell cycle effects were analysed. These results revealed that HCT116 cells under control conditions had a basal level of pre-G_1 cells of approximately 6% which remained for the duration of the experiment. The longer term effects of RHPS4 on the HT-29 cell cycle are very similar to those observed in the HCT116 cell line as we observed no difference in the proportion of pre-G_1 cells from those of the control at either 100 nM or 1 μM RHPS4. The proportion of cells in G_1/G_0 also remained relatively stable.

Analysis of the cell cycle profiles of KM12 cells in response to RHPS4 at concentrations of 100 nM, 1 μM and 10 μM revealed significant changes in the proportion of cells in pre-G_1. It was evident that at the higher concentration of RHPS4 analysed, there were no significant effects on the proportion of cells in G_1/G_0, S or G_2/M. As stated previously, the HCC-2998 cell line was the most unreliable with regards to the short term analysis of the cell cycle effects of RHPS4. It was also evident that this cell line was unreliable when analysing the longer term effects of RHPS4. There was an increase in the proportion of cells in pre-G_1 and G_1/G_0 in a concentration-dependent manner, however, the results were irreproducible.

Based on the results from each cell line with regards to cell cycle, GI_{50} values and morphology, it was evident that the HCT116 cell line displayed the desirable

characteristics of the cell line which would be used for the development of a mathematical model. The HCC-2998 cell line, due to the irreproducibility of the cell cycle analysis, both short- and long-term and longer doubling time was no longer considered as a suitable cell line for the analysis of RHPS4.

It was desirable that the mathematical models incorporate as much information as possible with regards to the cell cycle, however, maintain simplicity to allow the practising biologist to utilise the model for the analysis of their data. In the laboratory, the standard experiment for analysing senescence is the SA-β-gal stain. Mammalian cells express a lysosomal β-galactosidase activity that has a pH optimum of 4.0 and cells which are in a state of replicative senescence express SA-β-gal which is measured at pH 6.0 and has become widely accepted as the biomarker for senescence. The SA-β-gal staining method is a cytochemical assay based on production of a blue-dyed precipitate formed from the cleavage of the chromogenic substrate X-gal. The numbers of blue cells were counted under the microscope to obtain a semiquantitative percentage of senescent cells. Although this is the standard method for analysing senescence, it is often left unattempted as the counting of stained cells is tedious, semiquantiative and potentially subjective (Bassaneze *et al*, 2008; Gary & Kindell, 2005). Analysing senescence in this way is controversial as Severino *et al* demonstrated that although SA-β-gal staining is increased under varying conditions, the interpretation of increased SA-β-gal staining with increased senescence remains unclear, as does the question whether the same mechanisms are responsible for increased SA-β-gal staining in senescent cells and changes observed in cells under other conditions (Severino *et al*, 2000). This method was, however, still used in this project. The cells were incubated with RHPS4 at concentrations equal to the GI_{25}, GI_{50} and GI_{75} for each individual cell line for up to 21 days and were analysed at days 4, 6, 10, 14 and 21. At the appropriate time, digital images of five random fields of view were taken and the proportion of stained cells was counted and an average obtained. The results showed that the morphology of KM12 cells made the counting of the proportion of senescent cells difficult as they grew in layers making it impossible to analyse the senescent cells in the lower layers and therefore making senescence analysis

difficult and inaccurate. The flat, monolayer morphology of HCT116 and HT-29 cells facilitated the analysis of senescence. The results revealed there was a concentration-dependent increase in the proportion of senescent cells evident in both HCT116 and HT-29 cells. From the evidence outlined in chapter 3, it was clear that the HCT116 cell was the most suitable for further investigation and the development of mathematical models of the cell cycle due to their ideal morphology for senescence analysis, short doubling time allowing a higher turnover of results, good sensitivity to RHPS4 with a GI_{50} value of 1 μM at 24 hours and reliability in reproducing observations.

7.3.2 Development of the Mathematical Models

Chapter 4 describes the development of the mathematical models starting with a very simple one-compartment model that realistically has no use other than to calculate the net rate at which the cells are proliferating. The aim was to derive a mathematical model of the cell cycle of adequate complexity to reproduce the biological results observed in the laboratory, yet sufficient enough to yield beneficial information and biological insights. It was also important to maintain the ODEs involved as simple as possible so that a practising biologist with no mathematical background could use the models to yield conclusions from their data. It was also important to ensure that the mathematical models must incorporate as much cell cycle compartmental data, senescence data and population doublings data as possible. Experimentally, we were able to derive data on the population doublings, proportion of cells in senescence and the proportions of cells in each phase of the cell cycle. Upon development of the mathematical models, it was obvious that the five-compartment model was the most compartments we could have and fewer-compartment models were tested but proved to not work well and therefore we concluded that the five-compartment model was the most accurate way in which we would incorporate all our data into a mathematical model and allow us to yield useful information regarding the effects of RHPS4 in HCT116 cells that would be difficult to appreciate without it.

7.3.3 Application of the Data to the Mathematical Models

In chapter 5 I explain the effects of RHPS4 on the cell cycle, population doublings and senescence of HCT116 cells over 21 days. The cells were analysed at days 4, 6, 10, 14 and 21 following incubation with RHPS4 at concentrations of 50 nM, 100 nM and 1 μM to determine the effect of time and concentration on HCT116 cells. The results revealed under normal growth conditions in the absence of RHPS4, there is a basal level of senescence of approximately 18±1.99% over the 21 days. RHPS4 induces a concentration- and time-dependent effect on senescence evident from the incubation with RHPS4 at a concentration of 50 nM at day 4 increases the proportion of senescent cells to 37.41±2.62% and increases further when incubated with RHPS4 at a concentration of 1 μM to 39.23% and by day 21 the effects are more apparent and there is an increase of the proportion of senescent cells of approximately 66% above the control. The cells were continuously subbed as they reached 60-70% confluence to maintain a logarithmic growth and were consistently exposed to RHPS4 to remove as many outside influences that may induce senescence as possible. RHPS4 has been reported as a known telomerase inhibitor and the treatment of cancer cells with these agents leads to progressive telomere shortening with no acute cytotoxicity, however, there is proliferation arrest after a lag period with the hallmarks of senescence (Damm *et al*, 2001). The SA-β-gal stain allowed the observation of this within our experiments in a concentration-dependent manner. Cells which are senescent become large and flattened and there is the presence of multiple vacuoles (Chen *et al*, 2005; Ren *et al*, 2008). This morphology was observed in HCT116 cells prior to staining. The cell cycle results reveal that RHPS4 perturbs the cell cycle, even at the lowest concentration of 50 nM, which induced no effects on the proportion of cells in pre-G_1, however, there did reduce the proportion of cells in the S and G_2/M phases of the cell cycle. Increasing the concentration of RHPS4 to 100 nM again has no effect on the proportion of cells in pre-G_1, however, reduces the proportion of cells in S and G_2/M. At the highest concentration of RHPS4 analysed, there a further increase in the proportion of cells in G_1/G_0 to 67.1±7.4%

and the concentration-dependent decreases in the proportion of cells in S and G_2/M were lost.

The overall growth rate of HCT116 cells in the presence and absence of RHPS4 was also observed to reveal a concentration-dependent decrease in the population doublings. Control cells underwent 13±0.97 doublings in 21 days which reduced to 11.5±1.0 in RHPS4 at a concentration of 50 nM and reduced further to 10.7±0.8 when the concentration was increased to 100 nM. At the highest concentration of RHPS4, the number of population doublings was reduced to 3.5±1.1 in 21 days and it appeared that after day 6 of the experiment the number of population doublings remained static and this was consistent in experimental repeats. Normal cells in culture will replicate until they reach a discrete point at which their population growth ceases. Telomerase inhibitors, such as RHPS4, reduce telomerase activity meaning the cells reach a critical telomere length more quickly than under normal circumstances and hence population growth ceases. It is possible that the experiments carried out as outlined in chapter 5 are not long enough for the changes in population growth of HCT116 cells in response to RHPS4 are due to telomere changes since 21 days is not long enough for telomere length to be significantly affected and so some other effect is causing a reduced population growth in a concentration-dependent manner (White *et al*, 2001). A study carried out by Leonetti *et al*, concluded that when used at higher doses, RHPS4 triggers a short-term apoptosis/senescence response in human melanoma cell lines and is correlated with an increased incidence of telomere fusions and suggests that this compound is more than a simple telomerase inhibitor. The effect of short-term apoptosis/senescence is not a result of telomere shortening and instead RHPS4 rapidly disrupts the telomere architecture without having any affect on telomere length and results in a potent DNA damage response (Leonetti *et al*, 2004; Salvati *et al*, 2007).

The data generated in the laboratory allows conclusions to be drawn regarding the emergent properties of RHPS4, however, does not allow the kinetics of the cell cycle to be examined and so lead more directly to precise molecular targets for RHPS4. The data was applied to the two-, three- and five-compartment models in

an attempt to determine the kinetics of the cell cycle and how they are affected with respect to concentration and time. The two-, and three-compartment models, as previously stated are unrealistic as they exclude the exit of cells from the system and so the data was applied to these models to illustrate that the conclusions regarding RHPS4 are different depending on the system of ODEs and therefore model used. The application of the data to the five-compartment model revealed interesting conclusions. The data for the individual concentrations was analysed in comparison to control cells as it was obvious from the data obtained from HCT116 cells under normal conditions there were changes in the rate parameters that should be accounted for and so the data generated with respect to the rate parameters k_{xy}, k_{yz}, k_{zx}, $k_{\Sigma A}$ and $k_{x\Sigma}$ were presented in comparison to control so that any changes revealed are changes that can be attributed to RHPS4. The results revealed a time-dependent increase in $k_{x\Sigma}$ in relative to the control. The same data reveals a decrease in $k_{\Sigma A}$, again relative to control though shows no time- or concentration dependence and instead reduces by day 4 and remains relatively static for the duration of the experiment. The data on the proportion of cells in each compartment reveals a concentration-dependent increase in the proportion of cells in the compartments A and Σ meaning that the presence of RHPS4 forces the cells into senescence and reducing the rate at which they die. In reality, cell death does not occur from senescence alone, and instead when conditions are unfavourable or DNA damage has been sustained, then the cells will undergo apoptosis. Apoptosis is a normal process in living organisms, first discovered over 100 years ago by the German scientist Carl Vogt in 1842. It is an evolutionary conserved method used to dispose of cells in an orderly fashion and there are several morphological features to apoptosis, including nuclear condensation and fragmentation, membrane blebbing and non-inflammatory phagocytosis. There are two pathways to apoptosis, namely extrinsic and intrinsic and conditions have to be in place for both to occur. The intrinsic pathway is triggered in the event of intracellular cues such as DNA damage, oncogene activation or hypoxia, whilst the extrinsic pathway is activated in the event of the activation of executioner caspases without the loss of mitochondrial potential

(Meyer *et al*, 2006). The apparent morphology of cells undergoing apoptosis is said to be similar to that of cells undergoing mitosis and suggests therefore that the biochemical changes could also be related. Unlike mitosis, however, apoptosis can occur during any phase of the cell cycle, as illustrated in figure 7.1 (King & Cidlowski, 1995). The checkpoints of the cell cycle link it to apoptosis and research over the past two decades has led to hundreds of genes which govern the initiation, execution and regulation of apoptosis to be identified (Yan & Shi, 2005). Data has also suggested that molecules which act in later G_1 of the cell cycle are needed by the cell for apoptosis (Meikrantz & Schlegal, 1995). It is possible that RHPS4 is having an effect on these events and therefore blocking the cells in a senescence state.

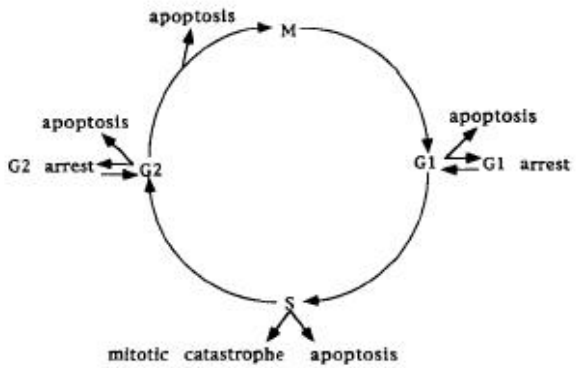


Figure 7.1 Pathways to apoptosis. Apoptosis, also known as programmed cell death, is a highly regulated process present to remove unwanted or damaged cells. Apoptosis can occur from any stage of the cell cycle when conditions are unfavourable (Taken from King & Cidlowski, 1995).

To maintain the simplicity of the ODEs in the five-compartment mathematical model, the value of $k_{\Sigma A}$ (i.e. the rate of cell death occurring from senescence) was considered to be much higher than the values of what would be k_{xA}, k_{yA} and k_{zA} (i.e. the rate of cell death occurring from G_1/G_0, S and G_2/M, respectively) and so were not accounted for. In addition to changes in $k_{x\Sigma}$ and $k_{\Sigma A}$, there is an increase in k_{xy} by day 21 in cells incubated with RHPS4 at concentrations of 50 nM and 100 nM but is not observed in those incubated with RHPS4 at a concentration of 1 μM. The mechanism of action of RHPS4 is very complex and is already known

to inhibit telomerase at submicromolar levels (IC_{50} = 0.33±0.13 μM) and exerts its chemotherapeutic potency through the interaction with and the stabilisation of four-stranded G-quadruplex structures (Gavathiotis *et al*, 2003). It is evident that RHPS4 displays time dependence, but not concentration dependence and therefore there are changes within the cells that are occurring straight away and effects that take longer to establish, and it is clear that it is these that are being modelled. At concentrations of RHPS4 of 50 nM and 100 nM, it is possible that we are observing many different kinds of effects within the cells and these are all being modelled, yet not being distinguished. At the higher concentration of 1 μM, we are 'blasting' the cell with a high dose and so we are possibly observing much simpler effects or the more dominant effects, hence the reason for the reduction in all rate parameters below the control except for the increase in $k_{x\Sigma}$. The five-compartment model is simply deterministic and allows us to draw conclusions based on experimental data. The model could be made more complex by splitting G_1/G_0 into two separate compartments; however, as we can't distinguish these easily experimentally as shown in chapter 3, we do not want to make the model more complex at this stage. At present we have mathematical models of varying difficulties based on the data we can generate and apply our data to and therefore determine conclusions. The mathematical models presented in chapter 5 are very simple, yet allow the data to speak for itself and are simply the starting point for the development of more realistic models with the concept of telomere length, age and where apoptosis occurs from. The models give preliminary insights into the mode of action of DNA-interactive agents, and whilst the mathematical models presented in chapter 5 do not resolve the mechanism of action of RHPS4, they allow further experiments to be more focussed and have allowed us to gain more knowledge in resolving the exact mechanism of action of this very complex molecule.

The five-compartment model was also challenged with a well-characterised drug, doxorubicin, to allow us to determine whether different conclusions are drawn. Doxorubicin cytotoxicity is cell cycle specific and at low concentrations is able to cause G_2 arrest without apoptosis (Demidenko *et al*, 2005). The application of the

data obtained from doxorubicin with regards to cell cycle, senescence and population doublings reveals that at each of the concentrations observed there is a reduction of each of the rate parameters in comparison to control at day 4, with the exception of cells incubated with doxorubicin at a concentration of 6.3 nM in which there is an increase in $k_{x\Sigma}$. Over time, the rate of each parameter recovers towards that of the control and by day 21 all rate parameters have increased above the control in a concentration-dependent manner, with the exception of $k_{\Sigma A}$. These conclusions are very different from those obtained from RHPS4 in the five-compartment model and show that the biggest decrease in comparison to control cells is observed in $k_{\Sigma A}$ and Demidenko *et al* observed that doxorubicin was able to cause G_2 arrest without causing cell death. We observed a reduction in the rate parameter k_{zx} in comparison to control, however, recovered by day 21 of the experiment and it is possible they could be explained in terms of resistance.

7.3.4 Simulation Based Analysis of the Mathematical Models

Chapter 6 outlines the application of the data that was derived from the five-compartment model to COPASI to essentially 'close the loop' and determine whether the results could be reproduced. COPASI is software application for the simulation and analysis of biochemical networks and was initially used to determine whether the results of doxorubicin were due to resistance, however was later proved not to be the case. The data obtained from the five-compartment model for RHPS4 was applied to COPASI and it was initially proven that the results could not be reproduced as what were being treated as rate constants, were in fact not constant and their values changed throughout the period of the experiment. This was also true for the rate parameter values obtained for doxorubicin. Further simulation and manipulation of the results allowed COPASI to reproduce the results of the five-compartment model. In order to allow COPASI to simulate the behaviour of both doxorubicin and RHPS4 in the five-compartment model, it was obvious there was more deviation from the actual values of the five-compartment model for RHPS4 than doxorubicin. In addition to this, doxorubicin displayed concentration- and time-dependent changes, whilst

RHPS4 displayed only time-dependent changes. It is evident therefore that whilst the mathematical models are useful, they fail to reproduce the behaviour of DNA-interactive agents with changing rate constants. It was also obvious that in the laboratory we were not observing exclusive linear growth, nor were we observing exclusive exponential growth. COPASI assumed exponential growth, and clearly identifies where the five-compartment model is failing.

7.4 Future Directions

The next approach in further investigating the five-compartment mathematical model as a useful tool for the investigation of anti-tumour agents would be to use other established anti-tumour agents, such as paclitaxel, and possibly in combination with RHPS4 to determine how this changes the outcome of the conclusions of the model with respect to RHPS4.

As indicated in chapter 1, POT1 is part of the shelterin complex and human POT1 controls telomerase-mediated telomere elongation and responds to the telomere length regulatory pathway governed by the TRF1 complex, acting downstream of TRF1 as a negative regulator of telomere lengthening (Loayza & de Lange, 2003; Ye *et al*, 2004). Molecules that dissociate POT1 from telomeres have cytotoxic activity and RHPS4 has been identified as one of these molecules, though does so indirectly (Salvati *et al*, 2007). RHPS4 was identified to cause damage foci at the telomeres and the overexpression of POT1 reduced damage foci and so concludes that overexpression of POT1 protects the cells from the cytotoxic activity of RHPS4 (Salvati *et al*, 2007). It is necessary that the biological studies outlined in chapters 3 – 5 are repeated in HCT116 cells whereby the knockdown of POT1 occurs, using siRNA and again analysing the results using the mathematical model and see how the conclusions compare. The knockdown of POT1 in HeLa cells has already proven successful and this induced a unique combination of telomere dysfunction phenotypes; diminished amounts of ss telomeric DNA and telomere-dysfunction-induced foci (TIFs), however, the cells continued to proliferate with no significant change in the number of telomere fusions observed (Hockemeyer *et al*, 2005). To keep in line with this study, an attempt at the knockdown of POT1

has already occurred in HCT116 cells, however, following several optimization methods, still remained unsuccessful, though it is necessary for the unequivocal knockdown of this protein in HCT116 cells in the further study of this complex molecule.

The five-compartment mathematical model, at present, assumes that cell death can only occur from senescence, however, the susceptibility of cells to apoptosis induction is influenced by their location in the cell cycle (Coquelle *et al*, 2006). Apoptosis results from a response to deregulated Myc expression and is capable of driving cells to die any stage of the cell cycle. Another body of evidence suggests that apoptosis occurs at the G_1-S border without apparent growth arrest (Kelley *et* al, 1994). Other studies indicate there is the arrest of the cells in G_1, without entry in S, with evidence suggesting DNA replication has already begun prior to arrest. In the human B cell lymphoma analogous to the murine WEHI-231 line, activation-induced apoptosis occurs from G_2 as opposed to G_1 (Ishigami *et al*, 1992). Generally speaking, it appears that cells must progress to late G_1 for apoptosis to occur as cell cycle arrest before this point delays or blocks the process of apoptosis, and arrest after this point promotes apoptosis and this is of significance since this is the p53 restriction point and progression of the cell through G_1 is in part regulated by p53 (Meikrantz & Schlegel, 1995). It is also possible for cell death to occur from mitosis as a result of mitotic catastrophe which is the combination of deficient cell cycle checkpoints and cellular damage. The inability of the cell to be arrested prior to mitosis triggers an attempt of aberrant chromosome segregation and activation of the apoptotic default pathways and cell death in mitosis (Castedo *et al*, 2004). It is important that this concept of cell death occurring from more than just senescence is incorporated into the mathematical model to further clarify the idea that there is an increase in the proportion of cells becoming senescent in the presence of RHPS4 relative to control cells and allow us to further understand the mechanism of action of this complex molecule as a telomerase inhibitor.