Devising Chemotherapeutic Treatment Regimen for Vincristine

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Abstract

Vincristine (VCR) is a core component of chemotherapeutic regimens for over half of pediatric malignancies, and has been used widely in anticancer therapy for more than 30 years. It is a key drug in the combination chemotherapy for childhood acute lymphoblastic leukemia (ALL) and non-Hodgkins lymphoma (NHL), however still very less is known about its side effects and optimal dosing regimen. Vincristine-induced peripheral neuropathy (VIPN) causes a peripheral, symmetric mixed sensory-motor, and autonomic polyneuropathy and is the dose-limiting toxicity. The clinical practice has been to use a one-size-fits-all approach of empirically capping the VCR at a total dose of 2 mg. However this practice may result in sub-therapeutic exposure and hence, compromised efficacy in a sizable population who may not be susceptible to VIPN. Full understanding of predictors of VIPN still remains elusive. The objective of this report is to identify a panel of metabolites that could provide valuable information to optimize VCR dosing without compromising efficacy or causing debilitating VIPN.

Keywords: Vincristine, Neuropathy, Metabolites

1. Introduction

Vincristine is one of the few clinically valuable extracts of the Catharanthus roseus (Madagascar periwinkle). Vincristine has a wide range of clinical applications and is a key drug in the combination chemotherapy for childhood acute lymphoblastic leukemia (ALL) and non-Hodgkinâ€™s lymphoma (NHL).It has been seen that response rates in hematological malignancies are generally higher than those in solid malignancies.

1.1. Single agent response rates

Single agent response rates in various malignancies are summarized in Table 1. However, these response rates should be interpreted with caution because of heterogenicity of patient populations and dosage regimens.

	Response percent
(A) *Childhood malignancies*	
ALL	76
ANLL	51
Hodgkin's disease	75
(A) *Adult malignancies*	
NHL	76
Hodgkin's disease	75

Table 1: Single agent activity of Vincristine in childhood and adult malignancies

1.2. Side effects

Vincristine has been know to cause both neural and non neural side effects. Mild and readily reversible myelosuppression with leucopenia and anemia have been described. Severe myelosuppression has been reported after overdosage. Thrombocytosis and Cellulitis with tissue necrosis have also been reported. However Neurotoxicity is the dose-limiting side effect of vincristine. Vincristine has been known to induce Sensory motor and Autonomic neuropathy in the early weeks, and as the time progresses has also been known to cause motor neuropathy. Recently, other late effects have been documented. Sensory neuropathy was found in one third of retrospectively studied adult patients up to 77 months after therapy was stopped. In a prospective study of children receiving vincristine without central nervous system irradiation, 50% had deep tendon reflex abnormalities, 20% gross motor and 30% fine motor disorders after a 2 - 3 year follow-up.

The following factors have been to known to affect neurotoxicity by vincristine:

- Dosage Regimen - Larger or more frequent doses than 1.4 mg/m^2 in adults and 1.5-2.0 mg/m^2 in children may result in unacceptable toxicity

- Age of the patient - Infants and adults appear to be affected most, but this apparent influence of age may, in fact, be due to previously inadequate dose calculation regimens
- Vincristine treatment in patients with obstructive liver disease is associated with an increased risk of neuropathy as vincristine effect might be potentiated by delayed biliary excretion
- More significant neurotoxicity has been described in patients receiving other therapy concomitantly

2. Hypothesis

Neurotoxicity introduces a problem for the dosage regimen. The empirical dosage cap is questioned due to wide inter-patient variability in tolerance and pharmacokinetics. This dosage cap limits the treatment of patients who may have a higher tolerance for the drug and at the same time may induce toxicity in patients with a lower tolerance limit. The problem then becomes to identify a set of features which can then be used to predict an appropriate dosage regimen and tolerance limit.

Hypothesis: The inherent metabolic profile contains sufficient information to predict metabolome and cellular response of drug treatment.

2.1. Rationale

The expression of a specific gene sequence into a final disease outcome proceeds at various levels. The genes are transcribed and translated into proteins which interact with the extracellular environment as enzymes, receptors and transporters. For each gene being translated into a protein variant alleles may exist, which change the pharmacokinetics of the drug. The resultant phenotypes at this level then exert phenotypic changes at the cellular, tissue and organ level through their influence on biological pathways. Variations in any of the steps mentioned above will result in changes at an organismal level. This translates into the possibility of observing patients with same genotype but different drug response, resulting in different treatment outcomes. Thus personalized treatment must not only consider DNA sequence and gene expression profiles related to drug metabolizing enzymes but it must also address their downstream implications. Hence biomolecules that are closely related to cellular response may provide vital information in predicting clinical outcome. Consequently small molecules (metabolites) qualify as the immediate effector of the clinical response.

2.2. Aims

- *Specific Aim 1:* To evaluate the association of pre-dose and post-dose metabolic profiles with VIPN and identify differentially expressed metabolites in pediatric patients

- *Specific Aim 2:* To develop a pharmacological model involving metabolites from SA1 to predict a carefully chosen VIPN score, thereby guiding the dosing decision support for optimizing VCR

2.3. Approach

Pharmacometabolomics is a field which stems from metabolomics, the quantification and analysis of metabolites produced by the body. Pharmacometabolomics refers to the direct measurement of metabolites in the body fluids, in order to predict the metabolism of pharmaceutical compounds, and to better understand the pharmacokinetic profile of a drug. Thus the field of pharmacometabolomics is directly involved with our research. We receive metabolite data from mass spectroscopy, and thus obtain a feature matrix and a binary response matrix. To select the necessary metabolites we would employ feature selection machine learning algorithms and then develop a model which can be used to predict neuropathy, and thus help in deciding dosage.

3. Feature Selection

We would be following the following convention from this point forward, the feature matrix is X matrix with the various columns representing the metabolite concentration in various datasets, the response matrix is Y matrix, a binary matrix indicating existence of neuropathy and the ID matrix is L matrix with the metabolite ID corresponding to columns of feature matrix.

We applied the following algorithms for feature selection:

3.1. Univariate Analysis

Univariate feature selection examines each feature individually to determine the strength of the relationship of the feature with the response variable. These methods are simple to run and understand and are in general particularly good for gaining a better understanding of data. There are lot of different options for univariate selection. We used the following three correlations:

- **Pearson Correlation:** Pearson correlation coefficient measures linear correlation between two variables. The resulting value lies in $[-1, 1]$, with -1 meaning perfect negative linear correlation, $+1$ meaning perfect positive linear correlation and 0 meaning no linear correlation between the two variables.

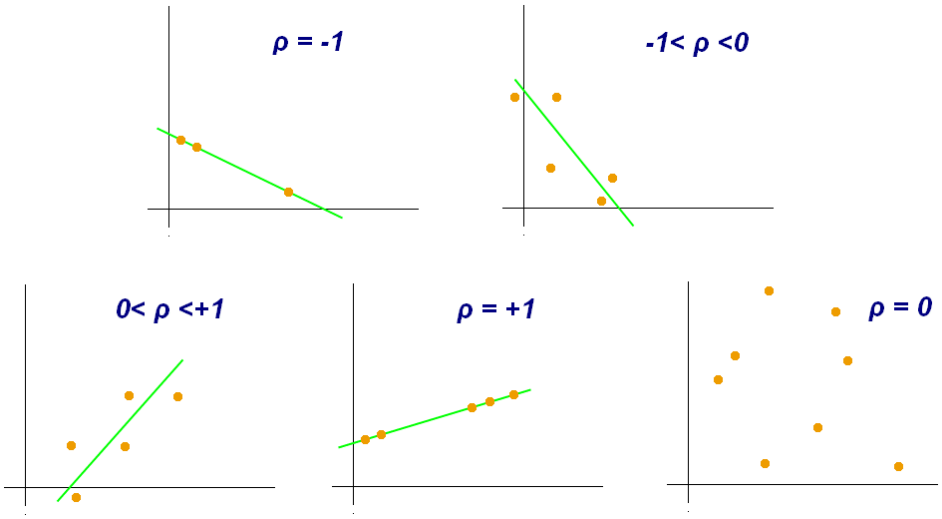


Figure 1: Scatter diagrams with different values of correlation coefficient

The formula for calculating pearson correlation coefficient is as follows:

$$\rho_{X,Y} = \frac{cov(X,Y)}{\sigma_X \sigma_Y} \tag{1}$$

where

- $cov(X,Y)$ is the covariance
- σ_X is the standard deviation of X
- X and Y are vectors

Pearson's correlation coefficient when applied to a sample is commonly represented by the letter r, and the formula can be written as:

$$r = r_{xy} = \frac{n\sum x_i y_i - \sum x_i \sum y_i}{\sqrt{n\sum x_i^2 - (\sum x_i)^2}\sqrt{n\sum y_i^2 - (\sum y_i)^2}} \tag{2}$$

- **Distance Correlation:** One drawback of Pearson correlation is that it is only sensitive to a linear relationship. If the relation is non-linear, it can be close to zero even if there is a 1 - 1 correspondence between the two variables. For example, correlation between x and x^2 is zero.

  Distance correlation is designed explicitly to address the shortcomings of Pearson correlation. Unlike Pearson correlation, a correlation value of 0 does imply independence in case of distance correlation.

  The distance correlation can be computed as:

$$dCor(X,Y) = \frac{dCov(X,Y)}{\sqrt{dVar(X)\, dVar(Y)}} \tag{3}$$

  where

  - $$dCov^2(X,Y) = cov(\|X-X'\|, \|Y-Y'\|) - 2cov(\|X-X'\|, \|Y-Y''\|) \tag{4}$$
  - $$dVar_n^2(X) := dCov_n^2(X,X) \tag{5}$$
  - X and Y are vectors
  - $E[X]$ represents the expectation value of X
  - X' is an independent and identically distributed copy of X
  - X'' is independent of X and X' and has the same distribution as X and X'

  For easy computation of sample distance correlation we use the *dcor* function in the *energy* package for R.

- **Maximal Information Coefficient:** Maximal Information Coefficient (MIC) is a measure of the strength of the linear or non-linear association between two variables X and Y. It was developed to remove the shortcomings of Mutual information Coefficient and it turns mutual information score into a metric that lies in range $[0, 1]$

  We use the *mine* function in the *minerva* package for R to calculate Maximal Information Coefficient.

3.2. Logistic Regression

Logistic regression is a regression model where the response is categorical. let the linear model be

$$p(X) = \beta_0 + \beta_1 X \tag{6}$$

if we fit a linear line to this this problem then there can always be some predictions $p(X) < 0$ and some $p(X) > 1$. To avoid this problem we model using a function that always gives values between 0 and 1. In logistic regression, we use the logistic function:

$$p(X) = \frac{e^{\beta_0+\beta_1 X}}{1 + e^{\beta_0+\beta_1 X}} \tag{7}$$

After a bit of manipulation we arrive at

$$\log\left(\frac{p(X)}{1 - p(X)}\right) = \beta_0 + \beta_1 X \tag{8}$$

The left-hand side is called the log-odds or logit.

3.3. Regularization using Lasso, Ridge and Elastic net regression

Classic linear regression solves the following problem

$$minimize \sum_{i=1}^{n} \left(y_i - \beta_0 - \sum_{j=1}^{p} \beta_j x_{ij}\right)^2 \tag{9}$$

In regularization we add a penalty factor and try to solve the problem

$$minimize\left(\sum_{i=1}^{n} \left(y_i - \beta_0 - \sum_{j=1}^{p} \beta_j x_{ij}\right)^2 + \lambda_1 \sum_{j=1}^{p} |\beta_j| + \lambda_2 \sum_{j=1}^{p} (\beta_j)^2\right) \tag{10}$$

with the following values of λ_1 and λ_2

- **Lasso regression:** $\lambda_2 = 0$
- **Ridge regression:** $\lambda_1 = 0$
- **Elastic net regression:** $\lambda_1 \neq 0$ $\lambda_2 \neq 0$
  The penalty factor is decided as: $\alpha\lambda_1 + (1 - \alpha)\lambda_2$, $\alpha\epsilon[0, 1]$

3.4. Decision Trees

A decision tree is a decision support tool that uses a tree-like graph or model of decisions and their possible consequences. The paths from root to leaf represents classification rules. One of the advantages of using decision trees is that they can be applies to both regression and classification problems.

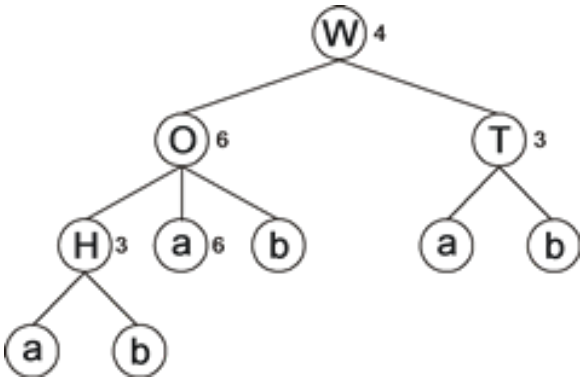


Figure 2: Decision Tree example

Bagging, random forests, and boosting use trees as building blocks to construct more powerful prediction models. Bagging involves creating multiple copies of the original training data set using the bootstrap, fitting a separate decision tree to each copy, and then combining all of the trees in order to create a single predictive model. Random Forests and Boosting further improve on bagging in the following way:

- **Random forests:** We build a number forest of decision trees on bootstrapped training samples. But when building these decision trees, each time a split in a tree is considered, a random sample of m predictors is chosen as split candidates from the full set of p predictors. The split is allowed to use only one of those m predictors. A fresh sample of m predictors is taken at each split, and typically we choose $m \approx \sqrt{p}$
- **Boosting:** Boosting works in a similar way as a bagging, except that the trees are grown sequentially, each tree is grown using information from previously grown trees.

4. Programming

The above algorithms for feature selection were applied to a test data of expression of lipids to test whether it is correlated to a disease stage of Non-alcoholic fatty liver disease (NAFLD). the results re presented below.

4.1. Univariate Analysis

We normalized the various correlations between $[0, 1]$ and then used that to select features. The criteria of important was set to select features whose correlation value was more than the median of the correlations vectors we obtained, for distance Correlation and Maximal Information Coefficient. This resulted in selection of 58 features. The importance plots for the top 20 predictors are:

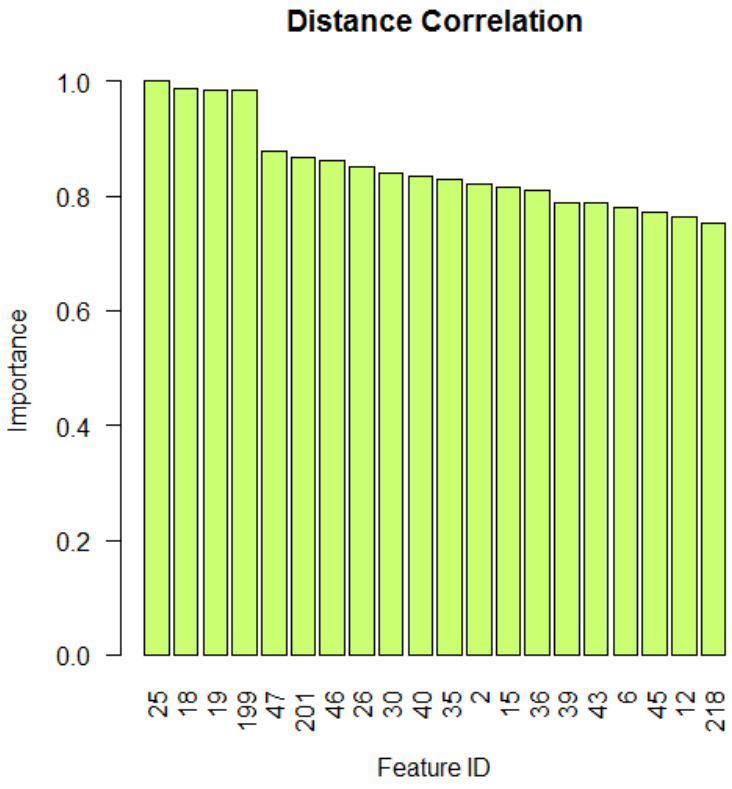


Figure 3: Feature selection from Distance Correlation

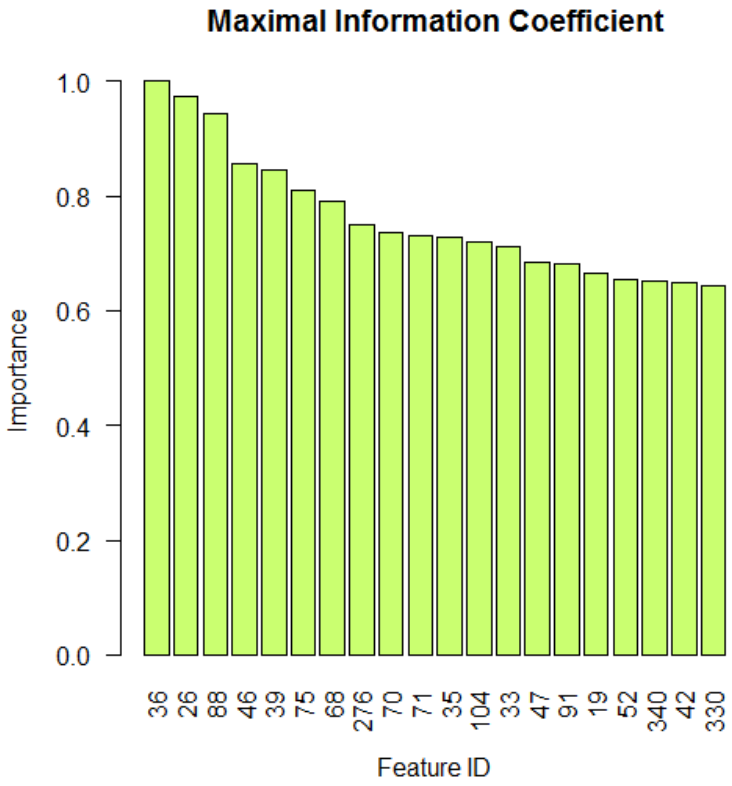


Figure 4: Feature selection from Maximal Information Coefficient

This limit of 58 features was then applied as the maximum number of features for all the algorithms

4.2. Regularization

All three regressions were allowed to run 100 times. The significant times were considered when the area under the ROC curve was found to be $>= 0.6$. Thus the frequency of all the features was noted. These frequencies were normalized on a scale of $[0, 1]$ and with an upper cap of 30 the important features were noted. The importance plots are as follows.

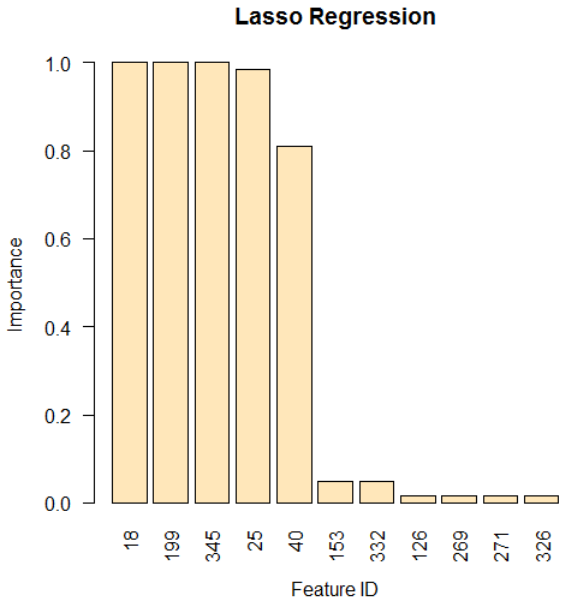


Figure 5: Feature selection from Lasso regularization

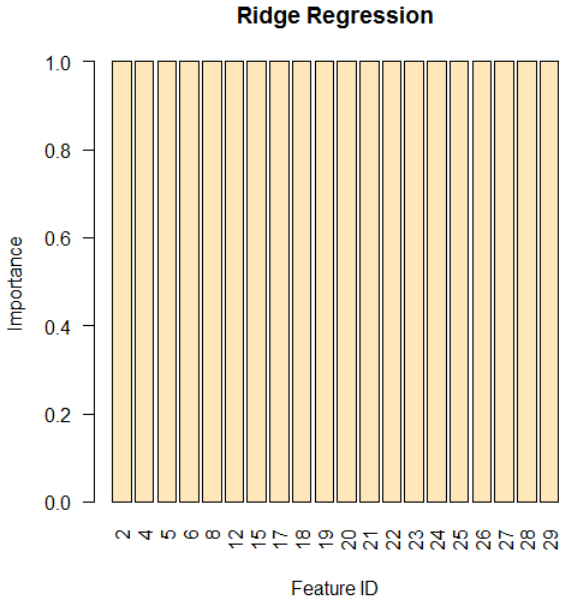


Figure 6: Feature selection from Ridge regularization

We see that the features have all been given importance of 1. This is because Ridge regression minimizes the quantity

$$minimise\left(\sum_{i=1}^{n}\left(y_i-\beta_0-\sum_{j=1}^{p}\beta_j x_{ij}\right)^2\right) \text{ subject to } \sum_{j=1}^{p}(\beta_j)^2 \leq s$$

Because $(\beta_j)^2$ is small Ridge has the tendency to select more features, though with very less value of β_j, in the order of 10^{-4}

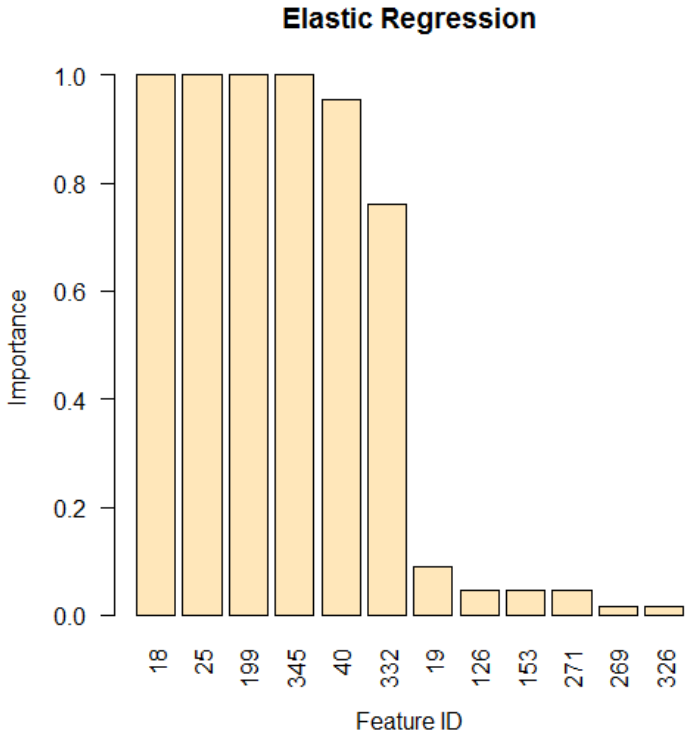


Figure 7: Feature selection from Elastic net regularization

4.3. Random Forest

Function *randomForest* was used from the library *randomForest* in R to implement random forests, and a gini plot of the the top 30 predictors was plotted.

The gini index is defined by

$$G = \sum_{k=1}^{K} \hat{p}_{mk}(1 - \hat{p}_{mk}) \tag{11}$$

where $\hat{p}_{mk}$ represents the proportion of training observations in the mth region that are from the kth class. A smaller value of gini index represents a more pure node.

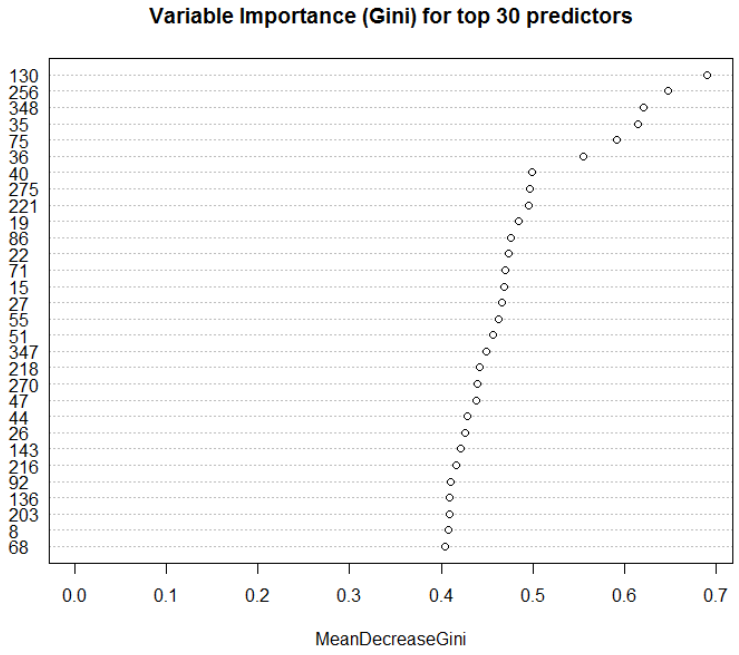


Figure 8: Gini index of predictors

4.4. Boosting

Function *xgboost* was used from the library *xgboost* in R to implement boosting, and the importance of the features was found using the function *xgb.importance*. The tree depth and number of rounds was found out by

varying these parameters over a whole range and then selecting the values which gave consistent results.

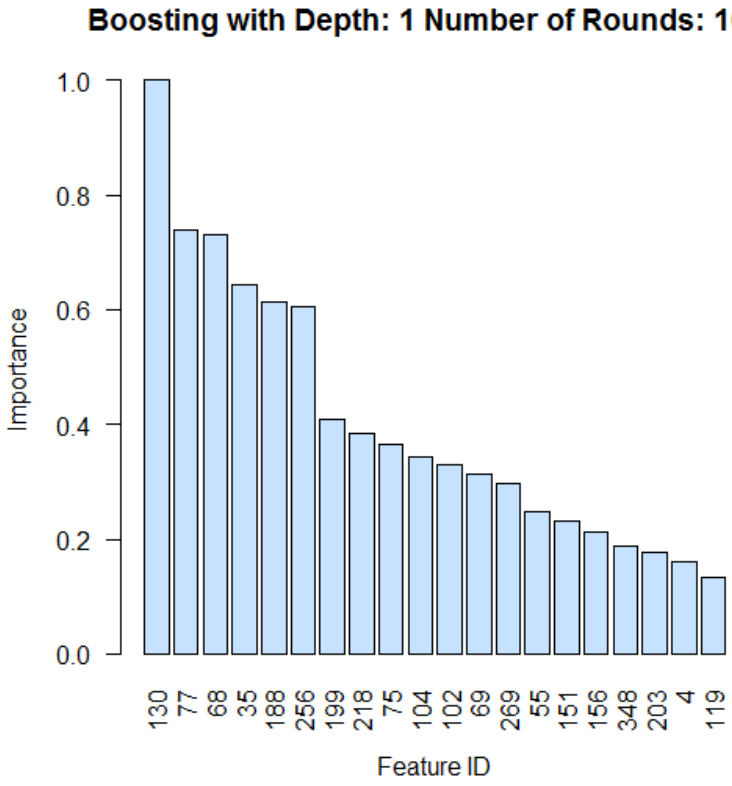


Figure 9: Top predictors from boosted Decision trees

4.5. Final Results

To select the final features the program gives the user to give each algorithm an importance rating in the range of $[0, 1]$, and based on this rating and the importance values from above algorithms the program displays the final features.

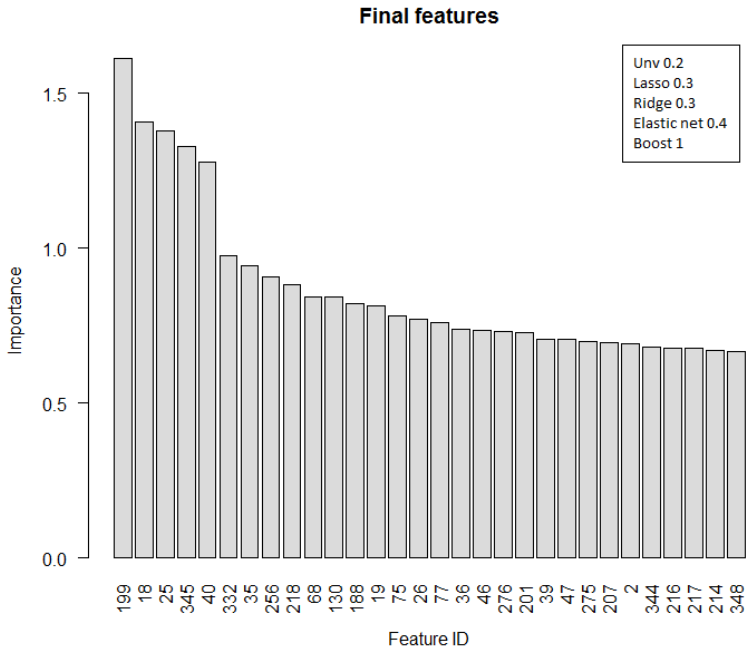


Figure 10: Final Selected features

We can see that all these features must be highly correlated and must significantly affect each other, from a biological point of view. Since Univariate Analysis and Regularisation fit the model without looking for effects from other features, we use Boosted Decision trees to build the final model, from the selected features.

5. Maximizing drug efficacy by timing drug dosage

Anti-mitotics are drugs that target microtubules to disrupt mitotic progression and are currently used exclusively for cancer treatment. It has been shown that when a cell is attacked during mitosis the following may happen:

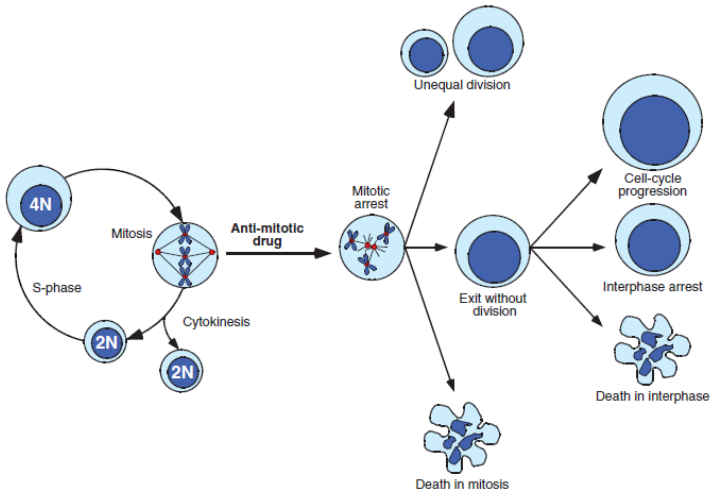


Figure 11: Cell fate in response to anti-mitotic drug treatment

This can be modelled as

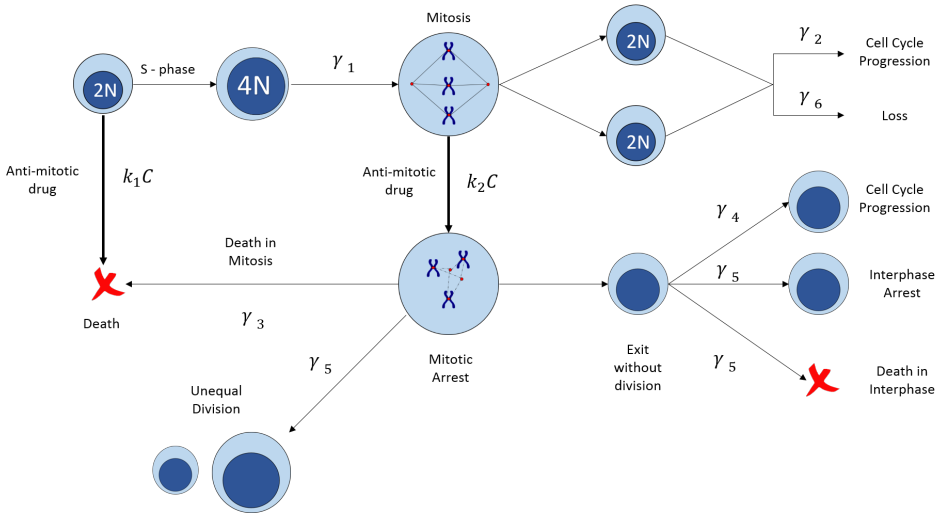


Figure 12: Pharmacodynamic model for drug attack

The following assumptions have been made:

- If an anti mitotic drug attacks in the G1 phase the cell dies immediately, if it attacks in any other phase the cell cycle continues till M phase
- If the cell remains unaffected till M phase it will divide into two cells and either continue once again with the cell cycle or leave the cell cycle. Currently in the code the loss term has been neglected
- If the drug attacks during Mitosis or the drug attack leads to M phase, the cell enters Mitotic Arrest
- If the cell enters Mitotic Arrest one of several things might happen, it may die instantly, it may divide unequally or it may exit without division
- It is assumed that if the cell undergoes unequal division it can no longer get back into cell cycle and is included in the loss term
- If the cell exits without dividing, it may once again start a cell cycle or enter Interphase Arrest or might die in Interphase, both of the latter terms are considered as a loss from the Mitotic Arrest

Based on the above assumptions the model can be reduced to Fig 14.

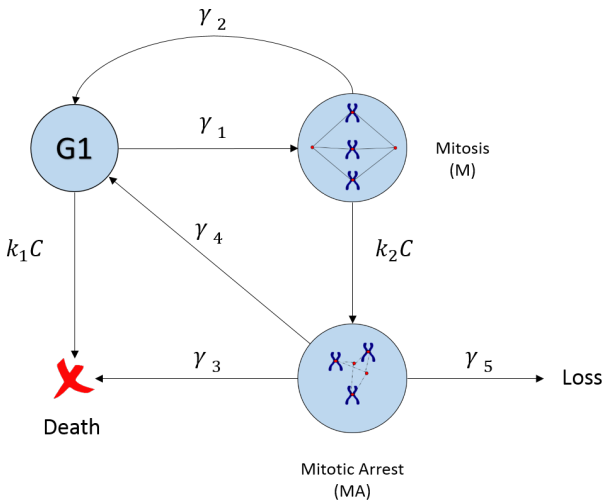


Figure 13: Working model

5.1. Population balance

The continuity equations for the different phases can be written as:

$$\frac{\partial n_{G1}}{\partial t} + \frac{\partial n_{G1}}{\partial \tau} + \gamma_1 n_{G1} + k_1 C n_{G1} = 0 \tag{12}$$

$$\frac{\partial n_M}{\partial t} + \frac{\partial n_M}{\partial \tau} + \gamma_2 n_M + k_2 C n_M = 0 \tag{13}$$

$$\frac{\partial n_{MA}}{\partial t} + \frac{\partial n_{MA}}{\partial \tau} + \gamma_3 n_{MA} + \gamma_4 n_{MA} + \gamma_5 n_{MA} = 0 \tag{14}$$

Here

- $n_i(t, \tau)$ represents age density function, where t is the time and τ is the time spent in i^{th} phase
- $N_i(t) = \int_0^\infty n_i(t, \tau)\, \partial\tau$,where $N_i(t)$ is the number of cells in i^{th} phase at a given time
- For the j^{th} dose of i^{th} drug

$$C_i(t) = \begin{cases} \frac{y_{ij}}{\lambda_i}(1 - e^{-\lambda_i(t - t_{AD,ij})}) + C_{i,residual}(t), & \text{during } j^{th} \text{ application} \\ C_{i,residual}(t) = \sum_{j=1}^{PA} \frac{y_{ij}}{\lambda_i} e^{-\lambda_i(t - t_{AD,ij})}(e^{\lambda_i h_{ij}} - 1), & \text{between applications} \end{cases}$$

  where

  - $C_i(t)$ is the concentration of i^{th} drug at time t
  - y_{ij} is the dose administration rate for the j^{th} dose of i^{th} drug
  - λ_i is the decay constant for i^{th} drug
  - h_{ij} is the dosage duration for the j^{th} dose of i^{th} drug
  - $t_{AD,ij}$ time when the j^{th} dose of i^{th} drug started

- Boundary conditions:
  - $n_{G1}(t, \infty) = 0, n_M(t, \infty) = 0, n_{MA}(t, \infty) = 0$
  - $n_{G1}(t, 0) = 2\gamma_2 \int_0^\infty n_M(t, \tau)\partial\tau + \gamma_4 \int_0^\infty n_{MA}(t, \tau)\, \partial\tau$
    $= 2\gamma_2 N_m(t) + \gamma_4 N_{MA}(t)$
  - $n_M(t, 0) = \gamma_1 \int_0^\infty n_{G1}(t, \tau)\, \partial\tau = \gamma_1 N_{G1}(t)$
  - $n_{MA}(t, 0) = k_2 C \int_0^\infty n_M(t, \tau)\, \partial\tau = k_2 C N_M(t)$

Integrating eqn (12), (13) and (14) with respect to $\partial\tau$ and applying the boundary conditions we get

$$\frac{dN_{G1}}{dt} = 2\gamma_2 N_M + \gamma_4 N_{MA} - (\gamma_1 + k_1 C)N_{G1} \tag{15}$$

$$\frac{dN_M}{dt} = \gamma_1 N_{G1} - (\gamma_2 + k_2 C)N_M \tag{16}$$

$$\frac{dN_{MA}}{dt} = k_2 C N_M - (\gamma_3 + \gamma_4 + \gamma_5)N_{MA} \tag{17}$$

5.2. Solution

Equations (15), (16) and (17) can be written as

$$\frac{d}{dt}(\mathbf{N}(t)) = \mathbf{AN} \tag{18}$$

where

$$\mathbf{N} = \begin{bmatrix} N_{G1} \\ N_M \\ N_{MA} \end{bmatrix} \qquad \mathbf{A} = \begin{bmatrix} -(\gamma_1 + k_1 C) & 2\gamma_2 & \gamma_4 \\ \gamma_1 & -(\gamma_2 + k_2 C) & 0 \\ 0 & k_2 C & -(\gamma_3 + \gamma_4 + \gamma_5) \end{bmatrix}$$

This is an eigenvalue problem with solution

$$\hat{\mathbf{N}} = \sum_{i=1}^{n} c_i e^{\lambda_i t} \hat{\mathbf{z}}_\mathbf{i} \tag{19}$$

where

- n is the number of eigenvalues of $\mathbf{A}$
- c_i is a constant
- λ_i is the i^{th} eigenvalue of $\mathbf{A}$
- $\hat{\mathbf{z}}_\mathbf{i}$ is the eigenvector corresponding to λ_i

To solve for eigenvalues we solve the equation $|\mathbf{A} - \lambda\mathbf{I}| = 0$, this would be a third degree polynomial equation in λ, giving three solutions. If only one root is real then the general form of these solutions is

$$\lambda_1 = k;\ \lambda_2 = a + ib;\ \lambda_3 = a - ib$$

$$\text{where } k, a, b\ \epsilon\ \mathbb{R},\ i = \sqrt{-1}$$

The corresponding eigenvectors are

$$\hat{\mathbf{z}}_\mathbf{1} = \begin{bmatrix} u \\ v \\ w \end{bmatrix}\ \hat{\mathbf{z}}_\mathbf{2} = \begin{bmatrix} x_1 + iy_1 \\ x_2 + iy_2 \\ x_3 + iy_3 \end{bmatrix} \text{ and } \hat{\mathbf{z}}_\mathbf{3} = \begin{bmatrix} x_1 - iy_1 \\ x_2 - iy_2 \\ x_3 - iy_3 \end{bmatrix} = \hat{\bar{\mathbf{z}}}_\mathbf{2}$$

The value of c_i was found by solving the following equation

$$\hat{\mathbf{N}}(t=0) = c_1\hat{\mathbf{z_1}} + c_2\hat{\mathbf{z_2}} + c_3\hat{\mathbf{z_3}} \tag{20}$$

Since L.H.S. is real and $\hat{\mathbf{z}}_\mathbf{3} = \hat{\bar{\mathbf{z}}}_\mathbf{2}$, we can say that $c_2 = \bar{c}_3$

Solving for $N_{G1}(t)$ we get

$$N_{G1}(t) = c_1\, e^{kt}\, u + c_2\, e^{(a+ib)t}\,(x_1 + iy_1)\ + c_3\, e^{(a-ib)t}\,(x_1 - iy_1)$$

$$N_{G1}(t) = c_1\, e^{kt}\, u + e^{(a+ib)t}\,(p + iq)\,(x_1 + iy_1)\ + e^{(a-ib)t}\,(p - iq)\,(x_1 - iy_1)$$

where

$$p = real(c_2) = real(c_3) \qquad q = imag(c_2) = -imag(c_3)$$

using $e^{(a+ib)t} = e^{at}(\cos(bt) + i\sin(bt))$ we get

$$N_{G1}(t) = c_1\, e^{kt}\, u + 2e^{at}\,(\cos(bt)\, px_1 - \cos(bt)\, qy_1 - \sin(bt)\, py_1 - \sin(bt)\, qx_1) \tag{21}$$

Hence we can say that if only one eigenvalue of $\mathbf{A}$ is real, then we would observe oscillations with time in the value of N_{G1}. Since N_M and N_{MA} also follow the same trend they would also show oscillations. This can help us in optimizing drug dosage as for maximum efficacy we can time the subsequent doses when the number of cancer cells show a maxima in G1, M or Mitotic Arrest phase.

The analytical and numerical solution to the equations was plotted in MATLAB. They are shown below in Fig. 15. The following values of parameters has been assumed

$$\gamma_1 = 1.1\ \gamma_2 = .01\ \gamma_3 = 11.2\ \gamma_4 = 1.02\ \gamma_5 = 1.4\ k_1 = 15\ k_2 = 1.1$$

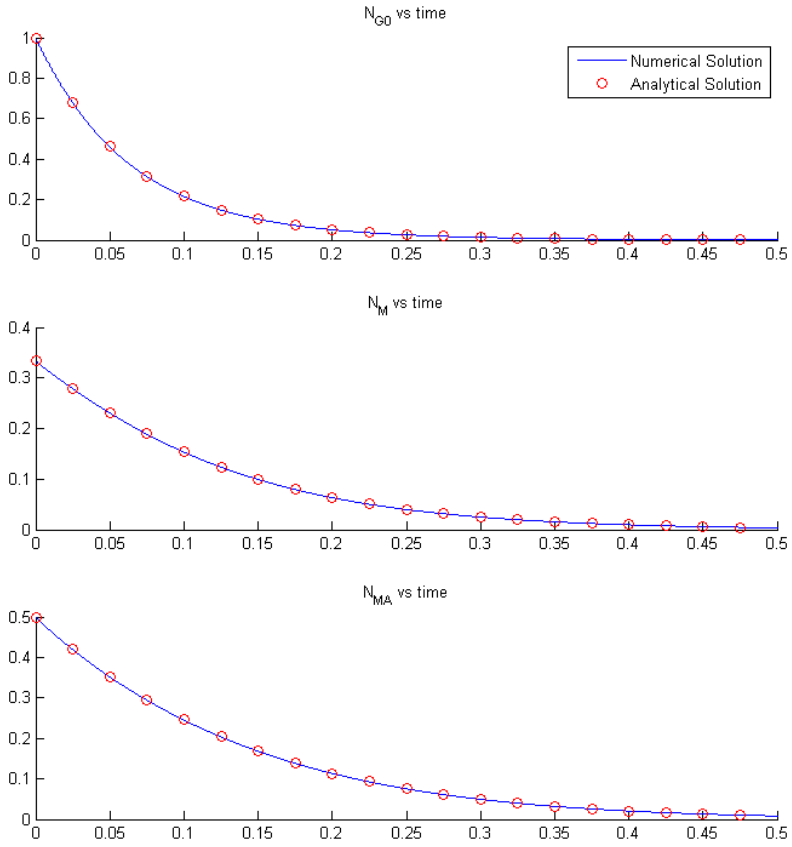


Figure 14: Solution

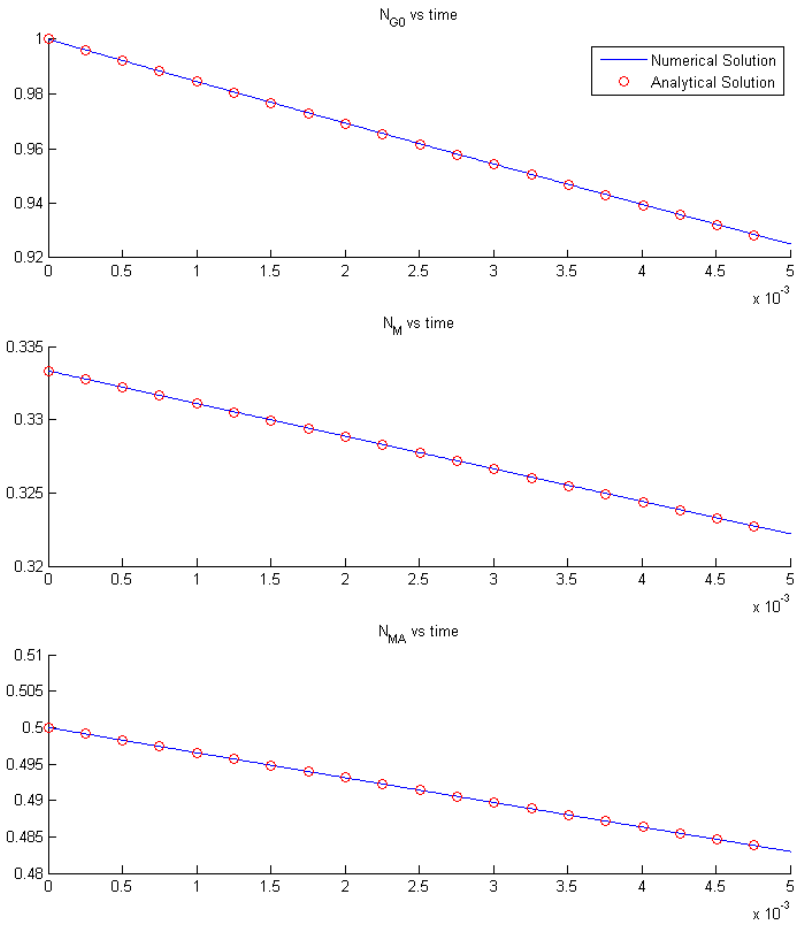


Figure 15: Solution with $C(t)$

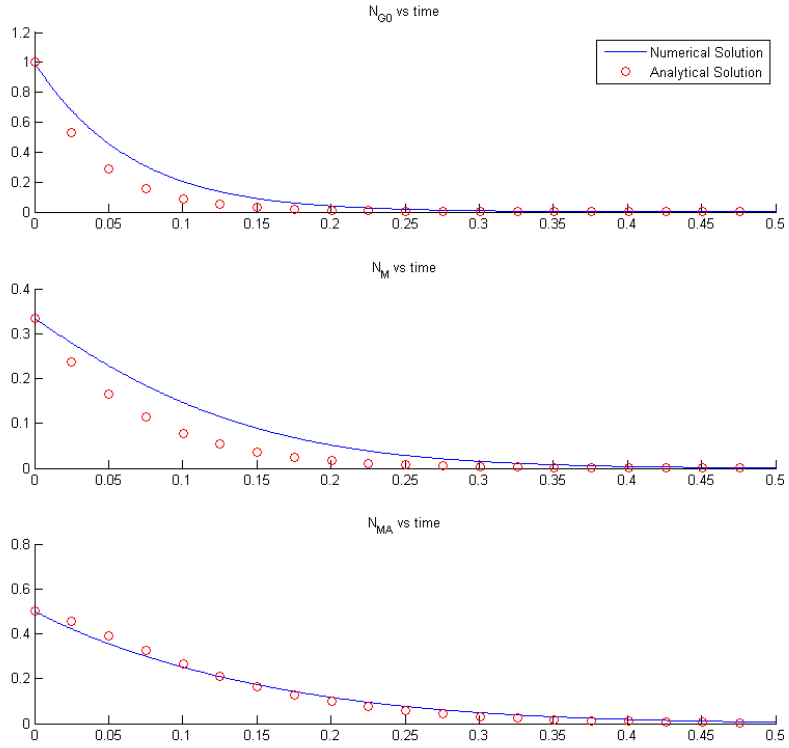


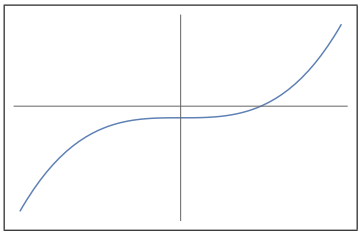
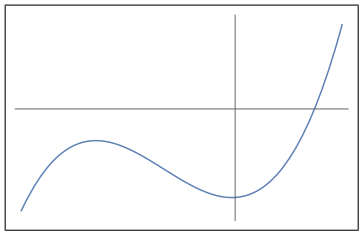
Figure 16: Solution with $C(t)$

5.3. Analysis

- The analytical and numerical solutions match exactly in Fig 14
- We don't see any oscillations, however it might be due to the fact that the amplitude of the oscillations is very small
- The graph shows decreasing concentration of number of cells in each phase with time, however this might be due to the fact that concentration of the drug has been taken as constant for Fig 14
- The plot when concentration of drug changes with time seems to have the same numerical and analytical solution for small period of time, but they change when we analyze over a larger time period

5.4. Challenges

- We want $|\mathbf{A}-\lambda\mathbf{I}|$ to show the following behavior: The equation $|\mathbf{A}-\lambda\mathbf{I}|$

is

$$-\lambda^3+\lambda^2(-\gamma_1+\gamma_2-\gamma_3-\gamma_4-Ck_1-Ck_2)+$$
$$\lambda(3\gamma_1\gamma_2-\gamma_1\gamma_3+\gamma_2\gamma_3-\gamma_1\gamma_4+\gamma_2\gamma_4-C^2k_1k_2-\gamma_1Ck_2+\gamma_2Ck_1-\gamma_3Ck_1$$
$$-\gamma_3Ck_2-\gamma_4Ck_1-\gamma_4Ck_2)-\gamma_3C^2k_1k_2-\gamma_4C^2k_1k_2$$
$$-\gamma_1\gamma_3Ck_2+\gamma_2\gamma_3Ck_1+\gamma_2\gamma_4Ck_1+3\gamma_1\gamma_2\gamma_3+3\gamma_1\gamma_2\gamma_4$$

With so many parameters it is difficult to control the behavior by guessing the values

- Moreover for eqn (21) to show oscillations the following conditions must be met

$$\text{(i) } 2\,(a+b)\,\sqrt{(\,px_1-qy_1)^2+(\,py_1+qx_1)^2}>k\,c_1\,u$$

$$\text{(ii) } a<0$$

Since some of these parameters are dependent on the eigenvectors and eigenvalues of $\mathbf{A}$, we do not have any direct control

- The analytical solution fails when we introduce time dependence
- Analysis with C changing with time was not possible because we were unable to obtain the desired form of eigenvalues
- It's entirely possible that if we guess the values we might end with a model which does not make biological sense

Hence without proper data about the rate constants, and knowing their time dependence it's pretty difficult to properly test our model

6. Conclusions

- With the help of our model one can predict a patient's susceptibility to VIPN, to decide proper dosage regimen
- The algorithms implemented can be applied for response prediction of many diseases
- Based on our model oscillations should be observed in the number of cells in M phase and G1 phase in cycle cell, and this could be used to time the doses for maximum efficacy
- Experimental data is required to verify our pharmacodynamic model for anti-mitotic drugs