Modeling and analysis of gene-therapeutic combination chemotherapy for pancreatic cancer

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Abstract: One of the most significant obstacles to successful chemotherapeutic treatments is the inevitable development of drug-resistant cells. Gene therapy techniques that seek to transfect chemotherapy susceptibility genes into the tumor have been tried, but these have met with little success, due to inadequate spread. Recent work has focused on exploiting dynamic evolutionary behavior by transfecting tumor cells with multidrug resistance gene 1 (MDR1) and thymidine kinase (TK), genes encoding a fitness advantage via chemotherapy resistance and a sensitivity to ganciclovir respectively. This process allows for positive and negative selection of the transgene creating a system with a natural optimal control formulation. We introduce an ordinary differential equation model of the interaction between susceptible and resistant tumor cells during periods of selection that arise as a result of drug treatment. We then develop a simple open loop optimal control system to demonstrate the necessity of a model based approach.

Keywords: Biomedical system modeling, simulation and visualization

1. INTRODUCTION

Numerous well known chemotherapy drugs, such as methotrexate and docetaxel, interfere with metabolic processes such as nucleic acid production (Banerjee et al. (1994)). These drugs effectively prevent reproduction. Some drugs trigger apoptosis mechanisms within cancers (Makin and Hickman (2000)). This mechanism exists to prevent genomically damaged cells from proliferating. However, the existence of malignant cancer cells is due to a loss or at least diminished function of native apoptosis triggers. This instability also frequently allows chemotherapy resistant mutants to arise. Many groups have considered the use of targeted gene therapy in order to reintroduce susceptibility to the mutant cells. Unfortunately, the spread of these susceptibility genes has never been able to encompass the entirety of the tumor; thus, gene therapy has largely been unable to affect the outcome post-treatment.

The recent work of Martinez-Quintanilla et al. (2009b), Martinez-Quintanilla et al. (2009a), seeks to overcome this problem using dynamic evolutionary processes. The proposed method of transfection calls for a hybrid gene that encodes an apoptosis trigger as well as a measure of chemoresistance. This allows for a phase of positive selection of transfected cells by simply applying the chemotherapeutic agent for which resistance has been gained to eliminate the remaining chemotherapy sensitive cells. This selection phase encourages spread of the susceptibility gene before introducing the corresponding apoptosis trigger, in this case, the common antiviral agent ganciclovir. If the spread of transfected genes has pervaded the tumor, bystander effects could be sufficient to cause death for all remaining cancer cell lines.

The dynamic nature of the model presents several significant issues. The ordinary differential equation models introduced explore the obstacles faced by this approach, such as efficacy and stability considerations. Furthermore, the cancerous cells with which we are concerned form solid tumors, likely resulting in non-negligible spatial effects. Using nonlinear logistic models of population growth, however, captures some of these spatial effects, and the ODE models used in this study should be sufficient for a preliminary investigation.

This paper is organized as follows: In Section 2, we provide a more detailed explanation of the treatment method from a biological standpoint. In Section 3, we introduce a basic model of competition between natural chemotherapy sensitive, natural chemotherapy resistant, and induced chemoresistant / antiviral susceptible cells. In Section 4, we examine the effects of a replication-competent delivery virus displaying various levels of virulence. We then look into an optimal control approach to maximize therapy effectiveness. Finally, in Section 5, we analyze the results and implications of our model for gene therapy.

2. BIOLOGICAL BACKGROUND

Chemoresistance Tumor cells are able to achieve states of chemoresistance via two mechanisms: mutation (natural evolution) or transfection of engineered genes. Natural mutations occur because cancer cell DNA is in a constant state of flux, continually altering their phenotype. Widely employed and well characterized chemoresistance genes are variants of dihydrofolate reductase (DHFR) and multidrug resistance gene 1 (MDR1) (Banerjee et al. (1994)).
DHFR serves as a catalyst for the reduction of folate to tetrahydrofolate (Simonsen and Levinson (1983)). This enzyme, in turn, serves as a cofactor in the production of amino and nucleic acids such as purines and thymidine. The chemotherapeutic agent methotrexate (MTX) is a compound having a greater affinity to DHFR than folate. Thus, DHFR more readily binds to MTX, preventing potential production of tetrahydrofolate and subsequent nucleotides. Ideally, methotrexate would display this characteristic for all tumor cells; however, DHFR mutants display a lower affinity for the drug, enabling them to continue preferential binding to folate.

Another instance of chemoresistance is illustrated by MDR1. This gene encodes for the membrane bound protein P-glycoprotein (PGP). The role of PGP is a facilitator of molecule movement, mediating both extra- and intercellular transport. PGP effectively acts as a drug pump, decreasing intercellular concentrations of drugs, thereby limiting their effectiveness.

Either of these mutations are considered highly likely to have an established presence in cases of advanced colon or pancreatic cancer. For the purposes of our model, we assume chemoresistant mutants constitute a portion of the tumor population. The selective pressure initiated by a chemotherapeutic agent favors these mutants, as they exhibit greater fitness than their non-mutant counterparts. Positive selection for mutants results in a post-treatment population that is devoid of chemo-susceptible cells.

Experimental anti-cancer gene therapy has been conducted on mice cancer cells, both in vitro and in vivo (Martinez-Quintanilla et al. (2009b), Martinez-Quintanilla et al. (2009a)). In both instances, cell populations were measured post-therapy, indicating a significant decline corresponding to successful treatment. However, the complicated dynamics of this process make it difficult to guarantee similar success in humans, especially when delivery mechanisms are considered. The remainder of this paper serves to demonstrate the necessity of dynamic modeling and optimal control techniques in designing a robust treatment implementation for human trials.

3. MODEL

Prior studies of anti-cancer gene therapy assume transfection is achieved by separately injecting a hybrid plasmid into natural chemosensitive cells (isolated from other cell lines). These cells are then reinserted to the tumor cell community along with the transfection. This is certainly a viable option for in vitro studies conducted on cell cultures; however, the sheer number of transfected cells required by anti-cancer therapy in humans, as well as time constraints, render this an impractical methodology. We will instead consider transfection via oncospecific viral vectors. We evaluate the feasibility of both replication-competent and replication-defective delivery viruses.

Non-Repli- cation Competent Delivery Virus Replication-defective viral vectors are capable of integrating with host cell DNA; however, the coding regions responsible for reproduction have been deleted. Thus, a replication-defective vector can infect a host, but is precluded from entering the lytic cycle that results in cell death. Inoculation of transfected genes by replication-defective viruses results in a fixed number of induced chemoresistant / ganciclovir sensitive cells, regardless of mitotic division. Such an instance would yield the system differential equations

\[
\begin{align*}
\dot{x} &= r x C_x(t) \left(1 - \frac{x + y + z}{K} \right) - x d_x + g(t) b \left(\frac{x + y + z}{x + y + z} \right) \\
\dot{y} &= \lambda y C_y(t) \left(1 - \frac{y}{x + y + z} \right) - y d_y + g(t) b \left(\frac{x + y + z}{x + y + z} \right) \\
\dot{z} &= s z C_z(t) \left(1 - \frac{x + y + z}{x + y + z} \right) - z d_z + g(t) \\
\end{align*}
\]  

where \(x(t)\) represents chemotherapy sensitive / ganciclovir insensitive cells; \(y(t)\) are chemotherapy resistant / ganciclovir insensitive cells; \(z(t)\) are the chemotherapy sensitive / ganciclovir sensitive cells. The exponential growth rates for each species are \(r, \lambda, s\), respectively, while \(d_x, d_y,\) and \(d_z\) are their natural death rates. The overall tumor growth is restricted by the carrying capacity \(K\).

\(C_x(t), C_y(t),\) and \(C_z(t)\) are inputs representing the application of chemotherapy, scaled according to its effect on each species’ growth. The application of ganciclovir is also an input, \(g(t)\), again scaled according to its efficacy, that acts primarily on the transfected cells. The bystander effect discussed in Section 2 is modeled as \(b(x, y + z)\), indi-

Transfected Genes In Martinez-Quintanilla et al. (2009b), Martinez-Quintanilla et al. (2009a), colon and pancreatic carcinoma cell lines are transfected via a plasmid containing one of two separate genes encoding either MDR1 or DHFR, combined with a gene encoding herpes simplex virus thymidine kinase (HSV-TK).

We have discussed the implications of MDR1 or DHFR presence in the cell. The second gene in the plasmid, HSV-TK, encodes an enzyme that converts the host to a state of susceptibility to the antiviral drug ganciclovir. Ganciclovir is prominent in many anti-cancer gene therapy approaches because it is readily incorporated into the DNA of susceptible cells. The compound is directly responsible for the formation of double-strand breaks in cell DNA, ultimately triggering apoptosis (Tomicic et al. (2002)). Furthermore, it diffuses into neighboring cells. This exchange of metabolized ganciclovir creates a bystander effect when transfected cells meet chemoresistant mutants at gap junctions, triggering apoptosis in cells not directly targeted by the drug.

The method of anti-cancer gene therapy under consideration occurs in a two-phase process. The first phase is positive selection for chemoresistant cells during chemotherapy, resulting in an amplified proportion of transfected cells. The population of transfected cells must outnumber that of mutant cells in order to ensure an optimal interaction between the species, required for the bystander effect. The second phase consists of negative selection phase achieved by injection of ganciclovir. Those cells prone to the antiviral drug treatment via transfection or bystander effect would undergo triggered apoptosis.

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cating that bystander effect increases as transfected cells become the dominant species.

Here, we have chosen to model the bystander effects as a monotonic function with properties similar to that of the sigmoid function. This is a dimensionless approximation of the likelihood that cells of type $x$ or $y$ are neighbored by cells of type $z$. This is a reasonable assumption; as the transfected cell population grows and approaches the carrying capacity of the tumor, $K$, it becomes inevitable that interaction with non-transfected cells will occur. For the purposes of this investigation and our ODE model, we have chosen to utilize a linear approximation of the generically sigmoid function $b$.

The derivation of our model is based on previous tumor models that have proven to be accurate for modeling solid tumor growth (Marusić et al. (1994a), Marusić et al. (1994b), Marusić et al. (1991), Komarova and D. Wodarz (2010)). The model assumes logistic tumor growth with a nominal set of parameters and a generic timeline.

**Chemotherapy System Effects** Initial conditions should reflect a tumor population that is predominated by chemotherapy sensitive cells. This is because, prior to the first introduction of chemotherapy, resistant cells have never enjoyed a selective advantage. Upon administering a chemotherapeutic agent, cells of subtype $x$ decrease dramatically, causing a corresponding reduction in tumor size. This is a temporary abatement as cells of subtypes $y$ and $z$ are positively selected by chemotherapy. Their continued growth yields a rebound in size as tumor growth remains encouraged.

Under the conditions of our model, subtypes $y$ and $z$ grow at an identical rate ($r = \lambda = s = 1$, $d_x = d_y = d_z = 0$, $C_1(t) = 0.95$, $C_y(t) = C_z(t) = 0$, $g_x(t) = g_y(t) = g_z(t) = 0$). Initial conditions: $x(0) = 90$, $y(0) = 6$, $z(0) = 5$.

Ganciclovir treatment affects sensitive and bystander effect-sensitive cells, causing a reduction in tumor size. Treatment will allow for a tumor size nadir that, if sufficiently small, would result in stochastic extinction of the entire tumor. Conversely, an insufficient bystander effect will result in a tumor size rebound shortly after ganciclovir treatment. At this point, only cells of subtype $y$ will remain; further treatments become impossible.

**Optimal Ganciclovir Timing** To avoid creating an entirely resistant tumor, we investigate the optimal timing of ganciclovir treatment. This will be observed as a continuous rise in the $y$ population, while $z$ cells will reach a maximum point before decaying. This is obviously not an ideal case; however to optimize bystander effects under these circumstances, ganciclovir should be administered at a point at which the population of $z$ reaches a maximum.

Alternatively, it is reasonable to assume that transfected genes may be engineered to grant a more significant degree of chemoresistance to subtype $z$. If transfected cells are more fit than natural chemotherapy resistant cells ($C_y(t) > C_z(t)$), we would observe identical dynamics to the previous case, with subtypes $y$ and $z$ simply exchanging roles. In this scenario, we would delay ganciclovir treatment for as long as possible (subject to toxicity) to capitalize on transfected cell growth and the bystander effect.

**Replication-Competent Delivery Virus** A more challenging, yet perhaps more beneficial approach is plasmid delivery by replication-competent viruses. In this manner, hybrid genes spread not only by mitotic division of transfected cells, but also through viruses capable of entering the lytic cycle. This would avoid the problem inherent in replication defective systems when GCV treatment fails, natural chemotherapy resistant cells rebound, and further transfections are impossible due to complete loss of hosts.

Yet this method is not without pitfalls; viral production is a multistep process of adsorption, replication of DNA, and finally, the destruction of host cells. This is beneficial only if the infection can spread through all cell lines, equally inhibiting the growth of all subtypes. Otherwise, as we will see, infected cell populations will peak, then decline once they have eliminated all susceptible hosts, leaving other subtypes to propagate freely.

We model transfection by replication-competent viruses by implementing a fourth state and additional parameters to our original model of equation 1.
\[
\dot{x} = rxC_x(t)(1 - \frac{(x + y + z)}{K}) - x[d_x + \beta v + g(t)b(\frac{z}{x + y + z})]
\]
\[
\dot{y} = \lambda yC_y(t)(1 - \frac{(x + y + z)}{K}) - y[d_y + \beta v + g(t)b(\frac{z}{x + y + z})]
\]
\[
\dot{z} = \beta xv + \beta vy + szC_z(t)(1 - \frac{(x + y + z)}{K}) - z[d_z + a + g(t)]
\]
\begin{align*}
\dot{v} &= kav - uv
\end{align*}

Here, we have included \(v\) as the viral population, containing the gene that will confer both chemoresistance and GCV susceptibility. Infections occur proportional to the mass action rates \(\beta_x\) and \(\beta_y\), depending on the interaction between the virus and non-infected cells. This constitutes a removal of the population from subtypes \(x\) and \(y\), and a growth in \(z\).

The rate \(a\) is the frequency of infected cell lysing required for viral proliferation. This rate depends on the virulence of the infecting virus; some will select for shorter latent periods, while others will cause cell destruction after a short post-infection delay. For every cell lysed, the burst constant \(k\) represents the number of free virus released. These viruses decay at the rate \(u\).

If the virus is able to infect cells of both type \(x\) and \(y\), then the infected cells will outcompete other subtypes strictly due to viral spread. This ratio of infected cells will yield a significant bystander effect upon introduction of GCV treatment, resulting in the removal of all cell types. However, a virus that is specific to subtype \(x\) will cause these cells to peak initially, while hosts are ample. Population declines in as subtype \(x\) cells reach stochastic extinction due to their high rate of transfer to chemoresistance. The genetic instability of naturally occurring mutants may make successful integration and transcription of the transfected gene problematic, so susceptibility of all cells to infection by the delivery virus should not be assumed. Depending on the virulence of the virus, this may necessitate a more dynamic treatment approach.

### 4. OPTIMAL CONTROL

In the case where natural resistant mutants are non-susceptible to infection, equation 2 should be amended to remove the effect of \(\beta_x\).

\begin{align*}
\dot{x} &= rxC_x(t)(1 - \frac{(x + y + z)}{K}) - x[d_x + \beta v + g(t)b(\frac{z}{x + y + z})] \\
\dot{y} &= \lambda yC_y(t)(1 - \frac{(x + y + z)}{K}) - y[d_y + \beta v + g(t)b(\frac{z}{x + y + z})] \\
\dot{z} &= \beta xv + \beta vy + szC_z(t)(1 - \frac{(x + y + z)}{K}) - z[d_z + a + g(t)] \\
\dot{v} &= kav - uv
\end{align*}

Using an oncospecific virus to execute transfection in the chemotherapy sensitive cells involves the consideration of a few parameters. A virus that spreads through the tumor very quickly would seem to be the obvious choice for virus selection. However, a replication competent virus that has a high infection will inevitably have a large burst size. An increase in burst size implies an increase in budding on the cell membrane leading to greater cell membrane damage and a higher virus-induced death rate. For this reason, the infection rate and virus-induced cell death rate are coupled in nature. Thus the options for what type of virus to select for treatment are between a fast spreading, high virulence delivery virus and a slow spreading, low virulence delivery virus.

The optimal time to introduce ganciclovir obviously occurs when the value \(\frac{z(t)}{x(t) + y(t) + z(t)}\) is at a maximum, leading to an optimal control formulation where we find a chemotherapy application schedule \(C_x\) satisfying

\[
\max_{t \in [0,T_{final}], C_x \in C} \frac{z(t)}{x(t) + y(t) + z(t)}
\]

subject to equation 3 and the given initial conditions, where \(C\) defines the set of clinically acceptable chemotherapy application schedules.

#### 4.1 Low Virulence Case

A low virulence delivery virus proliferates slowly in the tumor. The virus is able to spread in an optimal manner in this case. Chemotherapeutic sensitive cells die out as result of the chemotherapy treatment, rendering the more widespread transfected cells to outcompete the chemotherapy resistant cells for resources. As a result the transfected cells are able to reach total prevalence in the cell. This is most favorable, as the tumor can now be treated with the ganciclovir treatment and totally eradicated. This can be seen in Fig. 2.

The application of the chemotherapy causes the chemotherapy sensitive cells to crash as expected. While this is happening the virus begins to spread through the cell,
allowing the transfected cells to outcompete the rest of the cells. The natural chemotherapy resistant cells begin to rise initially but they quickly begin to decline as the transfected cells are becoming prevalent. One should note how slowly the natural chemotherapy resistant cells decline. Their decline is exponential in nature, but with a very long half-life, as it is driven by the competition for resources with the induced resistant cells; it will take a long time to completely decay without the intervention of treatment. Once the ganciclovir treatment is administered the transfected cells crash, Fig. 3, with the hope that as a result of the bystander effect the natural chemotherapy resistant cells also crash to zero. The trade off for a slow spreading, low virulence delivery virus is time. In this case the cost function is steady increasing toward the carrying capacity of the tumor. The optimal treatment is trivial in this case and would be to wait as long as possible before administering the ganciclovir. However, this is not practical because toxicity becomes a problem as chemotherapy exposure time increases.

**High Virulence Case** The opposite approach to the slow spreading, low virulence delivery is to instead employ the use of a fast spreading, high virulence delivery virus to transfect the cells. A high virulence delivery virus allows for rapid proliferation of infected cells, however due to the innate coupling of infection rate and burst size, the cells also have an increased viral death rate due to infection. Because of this, the transfected cells spread quickly, reach a high peak, and then begin to die out. Eventually if left untreated the natural chemotherapy resistant cells are able to outcompete the transfected cells leading to total dominance by the ganciclovir resistant cells in the tumor. For successful ganciclovir treatment the natural chemotherapy resistant cells must be totally wiped out as a result of the bystander effect.

The time \( t \) satisfying this maximum is the time where the peak dominance of the transfected cells occurs. The height of this peak, the point where the bystander effect occurs, the point where the bystander effect the peak dominance of the transfected cells occurs. The height of this peak, the point where the bystander effect the peak dominance of the transfected cells occurs. The height of this peak, the point where the bystander effect the peak dominance of the transfected cells occurs. The height of this peak, the point where the bystander effect the peak dominance of the transfected cells occurs. The height of this peak, the point where the bystander effect the peak dominance of the transfected cells occurs. The height of this peak, the point where the bystander effect.

**Initial conditions:**

\[
\begin{align*}
C_x(0) &= 0.95, \\
C_y(0) &= C_z(0) = 0, \\
g_x(0) &= g_y(0) = g_z(0) = 0.7, \\
\beta &= 0.0045, \\
a &= 0.27, \\
k &= 1.
\end{align*}
\]

**Parameter values:**

\[
\begin{align*}
C_x(t) &= 0.95, \\
C_y(t) &= C_z(t) = 0, \\
g_x(t) &= g_y(t) = g_z(t) = 0.7, \\
\beta &= 0.0045, \\
a &= 0.27, \\
k &= 1.
\end{align*}
\]

**Fig. 3. Low Virulence - Successful Therapy** Parameter values: \( C_x(t) = 0.95, C_y(t) = C_z(t) = 0, g_x(t) = g_y(t) = g_z(t) = 0.7, \beta = 0.0045, a = 0.27, k = 1. \) Initial conditions: \( x(0) = 60, y(0) = 10, z(0) = 10. \)

**Fig. 4. High Virulence Delivery Virus** Parameter values: \( C_x(t) = 0.95, C_y(t) = C_z(t) = 0, g_x(t) = g_y(t) = g_z(t) = 0.7, \beta = 0.0055, a = 0.33, k = 1. \) Initial conditions: \( x(0) = 60, y(0) = 10, z(0) = 10. \)

**Fig. 5. High Virulence Delivery Virus - Therapy Failure** Parameter values: \( C_x(t) = 0.95, C_y(t) = C_z(t) = 0, g_x(t) = g_y(t) = g_z(t) = 0.7, \beta = 0.0055, a = 0.33, k = 1. \) Initial conditions: \( x(0) = 60, y(0) = 10, z(0) = 10. \)

is most effective, varies with different treatments patterns of the chemotherapy. For this reason an optimal control method maximizing this peak will result in the best overall reduction in tumor size (and the best chance of total tumor extinction).

**Maximizing Cost Function** Our method of approach for optimizing chemotherapy treatment in order to maximize treatment effectiveness is the aforementioned control method, defined by equation 4. Using initial conditions of \( x_0 = 95, y_0 = 5, z_0 = 0, \) and defining \( C \) to be the set of schedules with chemotherapy being either fully applied \( C_x(t) = 0, \) or not applied \( C_x(t) = 1 \) at any given time. The schedules were allowed to switch no faster than a
given feasible switching time. We decimated our 50 day treatment schedule into 10 different intervals of 5 days with treatment in each interval either on or off. We then cycled through the $2^{10}$ possible chemotherapy treatment combinations and found which treatment schedule gave us the highest value for our cost interval. This allows the bystander effect to be maximized, because at the induced maximum point the percentage of transfected cells in the tumor is maximized. By introducing the ganciclovir at the time when this induced maximum is achieved, the optimal reduction in tumor size can also be achieved. This is shown in Fig. 6.

![Fig. 6. High Virulence Effect Parameter values: $C_x(t) = 0.95$, $C_y(t) = C_z(t) = 0$, $g_x(t) = g_y(t) = g_z(t) = 0$, $\beta = 0.0055$, $a = 0.33$, $k = 1$. Initial conditions: $x(0) = 80$, $y(0) = 10$, $z(0) = 10$. As Fig. 6 also shows, the optimal chemotherapy application may not always be a simple, one-dose schedule. Certain viral parameters and initial conditions may require chemotherapy applications with multiple timed pulses. This illustrates the necessity of a model-based approach to sequence design. This has previously been shown in other cases of combination chemotherapies involving gene therapies (Zurakowski and Wodarz (2006), Zurakowski and Wodarz (2007)). Once we have established what time this cost function is maximized we then stop the chemotherapy treatment and begin the ganciclovir treatment to totally eliminate the rest of the cells. The optimization is necessary in the case of a high virulence, quickly spreading virus because the bystander ratio will peak and then begin to decline. Through the use of optimal control techniques we can increase the peak value of the bystander ratio which would give a greater chance of successful therapy.

5. CONCLUSIONS

In this paper we discussed the application of dynamic modeling to a novel gene-therapeutic approach to pancreatic cancer treatment which relies on a dynamic positive selection process that would cause ganciclovir sensitive cells to spread optimally throughout the tumor, followed by a negative selection process. That would then allow the tumor to be totally eradicated.

Martinez-Quintanilla et al. (2009b), Martinez-Quintanilla et al. (2009a) conducted the proposed gene therapy experiments on mice to obtain in vivo therapy data. They were able to achieve a significant bystander effect but they were not able to totally eradicate the tumor. We believe that the results in our paper show how useful a model based treatment approach could be towards this problem and that future research in the area could contribute to therapy success. We acknowledge that our mathematical analysis is only preliminary, but it does serve to show how control techniques can be applied to the gene therapy problem. The promise of combination chemotherapies involving gene therapy is very high, but the implementation will likely be complicated. We have demonstrated a simple but effective optimal control method that could greatly increase the chances of a successful treatment.

REFERENCES


