MATHEMATICAL MODELING OF AN IMMUNE CHECKPOINT INHIBITOR AND ITS SYNERGY WITH AN IMMUNOSTIMULANT

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ABSTRACT. Immune checkpoint inhibitors (ICIs) are a novel cancer therapy that may induce tumor regression across multiple types of cancer. There has recently been interest in combining the ICIs with other forms of treatments, as not all patients benefit from monotherapy. We propose a mathematical model consisting of ordinary differential equations to investigate the combination treatments of the ICI avelumab and the immunostimulant NHS-muIL12. We validated the model using the average tumor volume curves provided in Xu et al. (2017). We initially analyzed a simple generic model without the use of any drug, which provided us with mathematical conditions for local stability for both the tumorous and tumor-free equilibrium. This enabled us to adapt these conditions for special cases of the model. Additionally, we conducted systematic mathematical analysis for the case that both drugs are applied continuously. Numerical simulations suggest that the two drugs act synergistically, such that, compared to monotherapy, only about one-third the dose of both drugs is required in combination for tumor control.

1. Introduction. Immune checkpoint inhibitors (ICIs) are a family of novel cancer therapies under investigation in multiple clinical trials. The checkpoint protein programmed death-1 (PD-1), mainly expressed on the surface of activated T cells, inhibits immune activation upon binding to its ligand programmed death-ligand 1 (PD-L1) [1]. Induction of PD-L1 on cancer cells and T cells is an important mechanism for tumor immune escape [7]. The development of drugs such as avelumab (PD-L1 inhibitor) and nivolumab (PD-1 inhibitor), that inhibit the binding of PD-1 with PD-L1, have shown promising results in treating different types of cancer.

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including melanoma and bladder cancer [1]. However, as reported in [30], up to 60 – 70% of patients fail to respond to ICI monotherapy. On the other hand, combination treatment may improve efficacy across tumor types and help address issues such as resistance [31]. Particularly, recent clinical trials suggest that coupling ICIs with treatments such as radiation, chemotherapy or other forms of immunotherapies, such as dendritic cell (DC) vaccines, improves the efficacy of the treatment [8].

Mathematical modeling has proven to be an important tool in cancer research, as it not only provides insights by capturing tumor mechanisms but also helps guide the design of new experiments based on predictions from the model [2]. Additionally, the incorporation of drug treatments in mathematical models can result in better understanding and improved treatments [23]. In recent years several mathematical models focusing on ICIs in conjunction with other treatments have been developed [11],[16],[19]. Specifically, Lai and Friedman [11] formulated a system of partial differential equations to study the efficacy and synergy between anti-PD1 treatment and a GM-CSF secreting cancer vaccine (GVAX), and created a synergy map of the recommended amount of drug dosages that should be administrated to reduce the tumor level. Radunskaya et al. [16] studied the effects of anti-PD-L1 in combination with a dendritic cell (DC) vaccine, tested against mouse data and investigated treatment schedules and effects of varying dosage amounts. Serre et al. [19] constructed a discrete pharmacokinetic model investigating synergism between radiotherapy anti-PD1/PD-L1 antibodies and/or CTLA4 inhibitor and used experimental data for validation.

While these models yield interesting results, we wish to capture the dynamics of applying a combination treatment to a continuous model in a more simplified manner than [11] and [16]. In [14] we developed a simple tumor-immune response model that incorporated the effects of a single immune checkpoint inhibitor (anti-PD-1) and mathematically analyzed the no drug case. Further global analysis of the mathematical model was presented in [21]. Using our work as a basis [14] and taking advantage of the data found in [29], we present a modification of the model and examine the effects of combining PD-L1 inhibitor with an immunostimulant. One important difference with the existing model is the use of the anti-PD-L1 antibody, avelumab. Avelumab is a monoclonal antibody that targets PD-L1, thus inhibiting its binding with PD-1 and enabling immune stimulation [4]. Additionally, we incorporate in the model an additional immunotherapy, NHS-muIL12. NHS-muIL12 is a fusion protein that binds to the DNA targeting the administration of IL-12 to damaged intratumoral regions [29].

In this work we initially propose a simple generic mathematical model that represents the dynamics of a tumor-immune response model without treatment. We mathematically analyze special cases of the generic model with the goal to build a both biologically and mathematically sound model that will describe the dynamics of a combination treatment based on the experiment in [29]. We use the mouse data from [29] to determine both appropriate biological, data-driven parameter estimates, and validate the model. Aside from the types of treatments used in the experiment [29], we also investigate the effects of continuous injection of both drugs (avelumab and NHS-muIL12). Finally, we numerically study the drug in combination to explore possible synergy between the drugs.
2. Formulation of mathematical model. In an effort to biologically capture the behavior of a tumor-immune response system that represents the data in [29] in the absence of treatments, we initially propose a simple generic mathematical model that follows a predator-prey structure. Examples of such mathematical models have been noted in several publications [13].

The initial model consists of two simple generic differential equations for the tumor cell volume \( V \) and the activated T cells volume \( T \) and has the following form:

\[
\frac{dV}{dt} = Vg(V) - p(V)T, \quad (1)
\]

\[
\frac{dT}{dt} = \delta F(V, T) - d_T T. \quad (2)
\]

We assume all functions appeared in the model are sufficiently smooth. In addition, we make the following more specific assumptions:

(A1): The initial tumor growth rate \( g(0) \) is positive and the growth rate \( g(V) \) is a non-increasing function of the tumor volume \( V \), as \( V \) approaches the maximum resources in the environment known as the carrying capacity.

(A2): The kill rate of the tumor cells \( p(V) \) is an increasing function of the tumor volume \( V \).

(A3): The T cell activation function \( F(V, T) \) is a positive and decreasing function of both variables.

### Table 1. State Variables of the model system (1)-(2) and (3)-(6).

| Variable | Meaning | Unit |
|----------|---------|------|
| \( V \)  | tumor cell volume | \( \text{mm}^3 \) |
| \( T \)  | volume of activated T cells | \( \text{mm}^3 \) |
| \( L \)  | free PD-L1 volume | \( \text{mm}^3 \) |
| \( P \)  | free PD-1 volume | \( \text{mm}^3 \) |
| \( A_1 \) | anti-PD-L1 concentration | g |
| \( A_2 \) | NHS-muIL12 concentration | g |
| \( Q \)  | PD-1-PD-L1 volume | \( \text{mm}^3 \) |

All the information regarding the variables of the generic model are described by Table 1. Equation (1) describes the tumor cell volume \( (V) \), where \( g(V) \) denotes the growth rate of the tumor volume. There is a variety of ways that the growth rate can be represented such as the logistic model \( g(V) = r(1 - \frac{V}{K}) \) with \( K \geq 0 \), the exponential model \( g(V) = r > 0 \), the Gompertz model \( g(V) = a - b \ln V \), and other forms [9]. The term \( p(V) \), kill rate of tumor volume by activated T-cells, is a function of the tumor volume \( V \) and can be represented by expressions such as \( p(V) = \eta V \) and \( p(V) = \frac{\eta V}{K + V} \). Equation (2) describes the activated T-cells volume \( (T) \), where \( F(V, T) \) describes the growth of activated T cells, and \( d_T T \) represents the natural death of the T-cells. Based on this generic model, we mathematically investigate two special cases of the model. The special cases consist of different tumor growth models, i.e logistic and exponential model. Both logistic and exponential growth models are commonly used to model tumor growth. A lot of work has been done that compares different growth models with the goal to achieve best fits as shown in [18]. Logistic growth describes tumor growth over a long period of time, since the tumor
has a limited amount of resources (carrying capacity) such as oxygen, nutrients to survive. Exponential growth has been widely used to model experimental data for early growth of the tumor [24]. We analyze these special cases of the generic model in the absence of treatments to gain mathematical and biological insights. These insights help us to build a special case model that best captures the synergistic effects of a combination treatment.

FIGURE 1. Schematic model representation for Tumor-Immune Interactions. Sharp arrows indicate proliferation/activation. Blocked arrows indicate killing/blocking. Dashed lines indicate proteins on the tumour (V) or T cells (T). IL-12 stimulates the proliferation of activated T-cells. NHS-muIL12’s role is to administrate IL-12 to damaged intratumoral regions. PD-L1 is mainly expressed on tumor cells. Anti-PD-L1 binds to PD-L1 to prevent the formation of the PD-1-PD-L1 complex.

We extend equations (1)-(2), using the previous work in [14], to develop a model of treatment response to two immunotherapies. Figure 1 represents a schematic network between all the model variables. The model consists of four nonlinear ordinary differential equations, which takes the following generic form:

1. \[
\frac{dV}{dt} = Vg(V) - p(V)T, \tag{3}
\]
   net tumor growth killed by T cells

2. \[
\frac{dT}{dt} = \delta F(V, T, A_1, A_2) - d_T T, \tag{4}
\]
   T cell activation death

3. \[
\frac{dA_1}{dt} = \gamma_1(t) - d_{A_1} A_1, \tag{5}
\]
   infusion rate of avelumab natural degradation

4. \[
\frac{dA_2}{dt} = \gamma_2(t) - d_{A_2} A_2, \tag{6}
\]
   infusion rate of NHS-muIL12 natural degradation

For the purpose of numerical simulations we choose the following functions:
\( g(V) = r, \)
\( p(V) = \eta V, \)
\[ F(V, T, A_1, A_2) = \left( 1 + \frac{\lambda_{T12}T}{\delta} \frac{c_2A_2}{K_{A_2} + c_2A_2} \right) \cdot \frac{1}{1 + \frac{Q(V, T, A_1)}{K_{TQ}}}. \]

All state variables of the system are described in Table 1. Equations (3) and (4) are the same as equations (1) and (2), where we choose exponential growth and a mass action term for the T-cell tumor killing. For the T cell activation function, \( F, \) we assume that NHS-muIL12 drug \( (A_2) \) activates T cells in a saturating manner, according to the Michaelis-Menten law. Note that \( c_2 \) represents a constant, where \( c_2 \in \left[ \frac{1}{75} \cdot 10^{-7}, \frac{1}{75} \cdot 10^{-6} \right] \) as derived in Section 9. As the amount of the complex PD-1-PD-L1 \( (Q) \) increases, T cell activation is reduced by a factor of \( \frac{1}{1 + \frac{Q}{K_{TQ}}} \).

The rate of change of avelumab \( (A_1) \) is modeled by Equation (5), where \( \gamma_1(t) \) is the prescribed infusion rate of avelumab. We consider the treatment protocol in [29], where avelumab is injected on days 0, 3, 6. The parameter \( d_{A_1} \) denotes the natural decay of \( A_1 \). Similarly to \( A_1 \), we consider a very simple differential equation for NHS-muIL12 \( (A_2) \), which is administered on day 0 as denoted by Equation (6). The parameter \( d_{A_2} \) depicts the natural decay of \( A_2 \). Moreover, we consider the case where we administrate both \( A_1 \) and \( A_2 \) continuously, and study the tumor dynamics for approximately 220 days.

What remains is to derive an expression for the complex PD-1-PD-L1 \( (Q) \) in equation (4). To that end, we start by observing that PD-1 \( (P) \) is expressed on the surface of activated T-cells [22],[20]. Hence, we take
\[ P = \rho_p T, \]
where, \( \rho_p \) is the cell rate of expression of PD-L1 on T cells as in [11].

PD-L1 \( (L) \) is expressed on the surface of activated T-cells and cancer cells [20]. Thus, the concentration of PD-L1 is proportional to T cells and cancer cells as in [11].
\[ L_{total} = \rho_L(T + \epsilon_v V), \]
where, \( \rho_L \) the cell rate of expression of PD-L1 on T cells and \( \epsilon_v \) depends on the specific type of tumor.

The amount of total PD-L1 consists of the amount of free PD-L1 and the amount of PD-L1 bound to the drug as described below.
\[ L_{total} = L_{free} + L_{bound}, \]
\[ L_{free} = L_{total} - L_{bound} = \rho_L(T + \epsilon_v V) - L_{bound}. \]
We assume the dissociation \( (d_Q Q) \) and association \( (\alpha_{PL}) \) of the complex PD-1-PD-L1 are fast so that we can apply the usual quasi-steady-state argument to the reaction as in [11].
\[ P + L \xrightarrow{\alpha_{PL}} \frac{1}{d_Q} Q. \]
We obtain \( \alpha_{PL} PL = d_Q Q, \) where \( \alpha_{PL} \) and \( d_Q \) are the association of \( PL \) and dissociation rate of \( Q, \) respectively. Thus, by equations (7) and (9)
\[ Q = \sigma PL_{free} = \sigma \rho_p T(\rho_L(T + \epsilon_v V) - L_{bound}), \] with \( \sigma = \alpha_{PL}/d_Q. \)
Next, we consider the reaction scheme:

\[ L_{\text{free}} + A_1 \overset{k_{+1}}{\underset{k_{-1}}{\rightleftharpoons}} L_{\text{bound}}, \]

where \( k_{+1} \) and \( k_{-1} \) are the constants for the forward and reverse reactions. The parameter \( k_{+1} \) describes the association reaction, and \( k_{-1} \) denotes the dissociation of anti-PD-L1 from the receptor. By the law of mass action and assuming the process is at equilibrium we have,

\[
\frac{dL_{\text{bound}}}{dt} = k_{+1}L_{\text{free}}A_1 - k_{-1}L_{\text{bound}} = 0,
\]

\[ L_{\text{bound}} = \frac{k_{+1}}{k_{-1}} L_{\text{free}}A_1 = \frac{k_{+1}}{k_{-1}} (L_{\text{total}} - L_{\text{bound}})A_1. \]

After solving for \( L_{\text{bound}} \) with algebraic manipulation, we conclude that

\[ L_{\text{bound}} = \frac{k_{+1}A_1}{k_{+1}A_1 + k_{-1}} (\rho_L(T + \epsilon_v V)). \]

Hence,

\[ L_{\text{bound}} = \frac{A_1}{A_1 + \frac{k_{-1}}{k_{+1}}} (\rho_L(T + \epsilon_v V)), \]

\[ L_{\text{bound}} = \frac{c_1 A_1}{c_1 A_1 + K_{A_1}} (\rho_L(T + \epsilon_v V)), \quad \text{with} \quad K_{A_1} = \frac{k_{-1}}{k_{+1}}. \quad (11) \]

Note that \( c_1 \) represents a constant, where \( c_1 \in [\frac{1}{55} \cdot 10^{-7}, \frac{1}{55} \cdot 10^{-6}] \) as derived in Section 9. By substituting equation (11) into equation (10), we have derived the following expression for \( Q \),

\[ Q = \sigma_P \rho_L T (T + \epsilon_v V) \left(1 - \frac{c_1 A_1}{c_1 A_1 + K_{A_1}}\right), \quad \text{with} \quad \sigma = \alpha P_L/d_Q. \quad (12) \]

Finally, using all the above derived equations we seek to gain a better understanding of the system (3)-(6).

3. Mathematical analysis for the general case. We initially consider the case when no drug is applied using the system of equations (1)-(2). We wish to determine some necessary conditions for the local stability of the tumorous and tumor-free steady states in the general form.

The general model has the following form:

\[
\frac{dV}{dt} = V g(V) - p(V)T, \quad (13)
\]

\[
\frac{dT}{dt} = \delta F(V, T) - d_T T. \quad (14)
\]

To facilitate the mathematical analysis, we make the following more specific assumptions:

1. \( g(V) \) is a continuously differentiable function and there exists a \( K > 0 \) such that \( g(V) > 0 \) when \( V \in (0, K) \) and \( g(V) < 0 \) for \( V > K \).
2. \( p(V) \) is a continuously differentiable strictly increasing function such that \( p(0) = 0 \).
3. \( F(V, T) \) is a continuously differentiable strictly decreasing, positive and bounded function.
We begin by studying the positivity and boundedness of solutions of system (13)-(14). The following proposition assures us that these desirable properties are held by system (13)-(14).

**Proposition 1.** Solutions of system (13) – (14) that start positive remain positive and bounded.

**Proof.** We first show that $T(t)$ can not be zero or negative before $V$. We assume that the starting time is $t_0$. Hence $T(t_0) > 0$. Then for $T(t) \leq 0$ for some $t > t_0$, there must be a time $t_1 > t_0$ such that $T(t_1) = 0$ and $\frac{dT}{dt}(t_1) \leq 0$. However, $\frac{dT}{dt}(t_1) = \delta F(V, 0) > 0$. Thus, $T(t) > 0$, for $t \geq t_0$.

Next we assume that there is a $t_1 > t_0$ such that $V(t_1) = 0$, and $V(t), T(t)$ are nonegative on $[t_0, t_1]$. By a standard separation of variables,

$$\frac{dV}{dt} = p(V) \left( V \frac{g(V)}{p(V)} - T \right) = p(V)(H(V) - T),$$

where, $H(V) = \frac{Vg(V)}{p(V)}$.

$$\frac{dV}{dt} = \frac{Vp(V)}{V}(H(V) - T),$$

$$\frac{dV}{V} = \left( \frac{p(V)}{V}(H(V) - T) \right) dt.$$  

We can conclude that,

$$V(t_1) = V(t_0)\exp\left[ \int_{t_0}^{t_1} \left( \frac{p(V)}{V}(H(V) - T) \right) dt \right] > 0$$

since $V(t_0) > 0$.

Hence, both $T(t)$ and $V(t)$ are positive when exist. Next, we would like to prove that they stay bounded.

We start with boundedness of $V$ using comparison argument.

$$\frac{dV}{dt} = Vg(V) - p(V)T$$

which implies that

$$V(t) \leq \max\{V(0), K\}.$$  

In addition, we see that

$$\lim_{t \to \infty} \sup V(t) \leq K.$$  

Therefore, $V$ is eventually bounded by $K + \alpha$, for any positive constant $\alpha$.

We then look to prove that $T$ is bounded. Since $V$ is bounded, then $F(V, T)$ is also bounded above by a positive constant, which we assume that it is $\gamma$. Hence

$$\frac{dT}{dt} = \delta F(V, T) - d_T T$$

which implies that

$$\lim_{t \to \infty} \sup T(t) \leq \frac{\delta \gamma}{d_T}.$$  

Similarly, we see that

$$T(t) \leq \max\left\{ T_0, \frac{\delta \gamma}{d_T} \right\}.$$
Hence, $T$ and $V$ are bounded. \hfill \Box

**Proposition 2.** System (13) – (14) has a unique tumor-free equilibrium $E_0^* = (0, T_0^*)$, $T_0^* > 0$.

*Proof.* Observe that $E_0^* = (0, T_0^*)$, $T_0^* > 0$ is an equilibrium of system (13) – (14) if and only if $\delta F(0, T_0^*) - d_T T_0^* = 0$. It is easy to see that $\delta F(0, T) - d_T T = 0$ has a unique positive solution since the function $\delta F(0, T) - d_T T$ is positive when $T = 0$, negative when $T$ is very large and is strictly decreasing when $T > 0$. \hfill \Box

**Proposition 3.** System (13) – (14) has a unique positive tumorous equilibrium $E^* = (V^*, T^*)$, $V^* > 0, T^* > 0$, if and only if $\delta F(V, H(V)) - d_T H(V) = 0$ has a unique positive solution in $(0, K)$, where $H(V) = \frac{V g(V)}{p(V)}$. In fact, $E_i = (V_i, T_i)$ is a positive equilibrium of system (13) – (14) if and only if $\delta F(V_i, H(V_i)) - d_T H(V_i) = 0$ and $v_i \in (0, K)$.

*Proof.* Setting $\frac{dV}{dt} = 0$ in equation (13) gives $T = H(V)$, where $H(V) = \frac{V g(V)}{p(V)}$. Then,

$$\frac{dT}{dt} = 0 \implies \delta F(V, H(V)) - d_T H(V) = 0. \tag{15}$$

Hence, system (13) – (14) has a positive equilibrium $E^* = (V^*, T^*)$, if and only if $V^*$ satisfies equation (15) and $V^* \in (0, K)$. This implies that the proposition is true. \hfill \Box

The next steps are to perform stability analysis for system (13) – (14). The Jacobian for the system written in terms of $H$ to facilitate further analysis is given by:

$$\begin{pmatrix}
    p'(V) (H(V) - T) + p(V) H'(V) & -p(V) \\
    \delta \frac{\partial}{\partial V} F(V, T) & \delta \frac{\partial}{\partial T} F(V, T) - d_T 
\end{pmatrix}.$$  

We examine the stability of both the tumor-free $E_0^* = (0, T_0^*)$ and tumorous equilibrium $E^* = (V^*, T^*)$. Observe that $\lim_{s \rightarrow 0^+} H(V) = g(0)/p'(0)$. For convenience, we define $H(0) = g(0)/p'(0)$. \tag{16}

**Proposition 4.** For system (13) – (14), the following statements are true.

(a) If $T_0^* > H(0)$, then $E_0^*$ is locally asymptotically stable.

(b) If $T_0^* < H(0)$, then $E_0^*$ is a saddle point.

*Proof.* The Jacobian matrix for the tumor-free equilibrium $E_0^* = (0, T_0^*)$, has the following form.

$$\begin{pmatrix}
    p'(0) (H(0) - T_0^*) & 0 \\
    \delta \frac{\partial}{\partial V} F(0, T_0^*) & \delta \frac{\partial}{\partial T} F(0, T_0^*) - d_T 
\end{pmatrix}.$$  

We know that $\frac{\partial}{\partial V} F(V, T) < 0$ and $\frac{\partial}{\partial T} F(V, T) < 0$ by assumption, and that the eigenvalues are of the form:

$$\begin{align*}
    \lambda_1 &= p'(0) (H(0) - T_0^*) < 0, \text{ if } T_0^* > H(0), \\
    \lambda_2 &= \delta \frac{\partial}{\partial T} F(0, T_0^*) - d_T < 0.
\end{align*}$$

Thus, $E_0^*$ is locally asymptotically stable if $T_0^* > H(0)$ and a saddle point if $T_0^* < H(0)$. \hfill \Box
The next proposition deals with the stability of a tumorous equilibrium.

**Proposition 5.** For system (13)−(14), the following statements are true:

(a) If \( H'(V^*) < 0 \) and \( H'(V^*) \left[ \delta \frac{\partial}{\partial T} F(V^*, T^*) - d_T \right] + \delta \frac{\partial}{\partial V} F(V^*, T^*) > 0 \), then \( E^* = (V^*, T^*) \) is stable.

(b) If \( H'(V^*) > 0 \) or \( H'(V^*) \left[ \delta \frac{\partial}{\partial T} F(V^*, T^*) - d_T \right] + \delta \frac{\partial}{\partial V} F(V^*, T^*) < 0 \), then \( E^* = (V^*, T^*) \) is a saddle point.

**Proof.** The Jacobian matrix for both parts (a) and (b) for the tumorous equilibrium \( E^* = (V^*, T^*) \) has the following form.

\[
\begin{pmatrix}
 p(V^*) H'(V^*) & -p(V^*) \\
 \delta \frac{\partial}{\partial V} F(V^*, T^*) & \delta \frac{\partial}{\partial T} F(V^*, T^*) - d_T 
\end{pmatrix}
\]

Then the trace for the tumorous equilibrium is

\[
\tau = p(V^*) H'(V^*) + \delta \frac{\partial}{\partial T} F(V^*, T^*) - d_T.
\]

It is easy to see that the trace is negative when \( H'(V^*) < 0 \) by condition in part (a).

The determinant is given by

\[
\Delta = p(V^*) \left\{ H'(V^*) \left[ \delta \frac{\partial}{\partial T} F(V^*, T^*) - d_T \right] + \delta \frac{\partial}{\partial V} F(V^*, T^*) \right\}. 
\]

The determinant is positive provided

\[
H'(V^*) \left[ \delta \frac{\partial}{\partial T} F(V^*, T^*) - d_T \right] + \delta \frac{\partial}{\partial V} F(V^*, T^*) > 0.
\]

Hence, the tumorous equilibrium \( E^* = (V^*, T^*) \) is stable provided the condition in part (a) is true. Similarly, the conclusion of part (b) follows. \( \Box \)

**Proposition 6.** If \( H'(V) < 0 \) for \( V > 0 \), then system (13)−(14) has no nontrivial positive periodic solutions.

**Proof.** We employ the Dulac criterion to show there are no nontrivial positive periodic orbits. Let \( b(V) = 1/p(V) \). Let \( F_1(V, T) = \frac{V p(V)}{V} (H(V) - T) \) and \( F_2(V, T) = \delta F(V, T) - d_T T \). Then it is easy to see that

\[
\Omega = \frac{\partial [b(V) F_1(V, T)]}{\partial V} + \frac{\partial [b(V) F_2(V, T)]}{\partial T} = H'(V) + \frac{1}{p(V)} \frac{\partial [b(V) F_2(V, T)]}{\partial T} < 0.
\]

By the Dulac criterion, we see that the system (13)−(14) has no nontrivial positive periodic solutions. \( \Box \)

With arguments similar to that of the proof of Theorem 3.7 in [14], we can obtain the following global stability result for the tumor free equilibrium in system (13)-(14). We refer readers to [14] for details that may be used for constructing a rigorous proof.

**Theorem 3.1.** If \( H'(V) < 0 \) for \( V > 0 \) and \( \delta F(V, H(V)) - d_T H(V) = 0 \) has no positive solution, then the tumor free equilibrium \( E^*_0 = (0, T^*_0) \) in system (13)−(14) is globally attractive with respect to positive solutions.
Clearly, the above results can be applied directly to the case of logistic growth. In fact, the local stability analysis for the logistic growth model was presented in [14].

The exponential growth case can be regarded as the limiting profile for logistic growth when the carrying capacity is set as infinity. With this in mind, we can apply the conditions found above to the exponential growth model. Since the exponential growth case will be the focus of the treatment study, we present the following specific local stability results for the case with \( g(V) = r \), \( p(V) = \eta V \) and

\[
F(V, T) = \frac{1}{1 + \alpha'T(T + \epsilon_v V)}, \tag{17}
\]

where \( \alpha' = \frac{\sigma\rho\rho_l}{\theta_1} \). We present them as corollaries. Proofs of the corollaries follow directly from Propositions 4 and 5.

**Corollary 1.** For the tumour-free equilibrium \( E^*_0 = (0, T^*_0) \), the following statements are true.

(a) If \( r < \eta T^*_0 \), then \( E^*_0 \) is locally asymptotically stable.
(b) If \( r > \eta T^*_0 \), then \( E^*_0 \) is a saddle point.

The condition \( r < \eta T^*_0 \) can be biologically interpreted, as the fact that \( T \) cells killing rate is faster than the growth rate of cancer cells.

**Corollary 2.** The tumorous equilibrium \( E^* = (V^*, T^*) \) is a saddle point.

Now we wish to explore the dynamics of the model (3)-(6), when both drugs are applied continuously as shown in Section 9 and the dynamics of an approximation of the system (3)-(6) as described below.

### 4. Mathematical analysis for the continuous treatment case: Limiting system.

We consider the differential equation system (3)-(6) and examine the case when both anti-PD-L1 treatment and NHS-muIL12 treatment are applied continuously. In this section, we assume that \( g(V) = r \), \( p(V) = \eta V \) and (17) holds. We wish to obtain necessary conditions for the local stability of the tumorous and the tumour-free steady states. We perform mathematical analysis for the full system (3)-(6) in Section 9. As the full system is complicated to perform extensive mathematical analysis on, we consider applying a quasi-steady state approximation. We let \( A_1 \) and \( A_2 \) go to quasi-steady state. The reduced system consists of a set of positive differential equations and takes the following form:

\[
\frac{dV}{dt} = rV - \eta VT, \tag{18}
\]
\[
\frac{dT}{dt} = \left( \delta + \lambda_{T12}TM \right)F(V, T) - d_T T, \tag{19}
\]

where,

\[
M = \frac{c_2 \gamma_2}{d_{A2} K_{A2} + c_2 \gamma_2},
\]
\[
N = \frac{c_1 \gamma_1}{d_{A1} K_{A1} + c_1 \gamma_1},
\]
\[
Q = \sigma\rho\rho_l T(T + \epsilon_v V)(1 - N),
\]
\[
F(V, T) = \frac{1}{1 + \alpha'T(T + \epsilon_v V)}. 
\]
\[ \alpha' = \frac{\sigma \rho_p \rho_l}{K_{TQ}} (1 - N), \quad \text{with } N < 1. \]

Due to the fact that the function \( \left( \delta + \lambda_{T_{12}} T M \right) F(V,T) \) may not be decreasing with respect to \( T \), we see that system (18) – (19) is not a special case of system (13) – (14). It is easy to see the solution of the above system (18) – (19) with positive initial values is positive. We seek to examine conditions ensuring boundedness of solutions for the system. Due to the assumption of an exponential growth form for \( V \), we see that \( V \) can grow exponentially in the absence of \( T \). Hence, for any constant \( M > 0 \), we can find a solution with small positive initial values in both \( V \) and \( T \) that can have values of \( V \) exceed \( M \) at some later time. Therefore, we are not able to establish boundedness for \( V \). However, the following proposition is true.

**Proposition 7.** The \( T \) component of any positive solution of system (18) – (19) is bounded.

**Proof.** System (18)-(19) is equivalent to

\[
\begin{align*}
\frac{dV}{dt} &= rV - \eta VT \\
\frac{dT}{dt} &= \left( \frac{1}{1 + \alpha'} \right) \frac{1}{T + \epsilon_v V} - d_T T.
\end{align*}
\]

It is easy to see that for positive values of \( V \) and \( T \), the function \( \left( \delta + \lambda_{T_{12}} T M \right) \frac{1}{1 + \alpha'} \frac{1}{T + \epsilon_v V} < \gamma \) for some positive constant \( \gamma \). This implies, that \[
\frac{dT}{dt} < \frac{\gamma}{d_T} - d_T T.
\]

By comparison argument, we see that \( T \leq \max\{ T_0, \frac{\gamma}{d_T} \} \) and \[
\lim_{t \to \infty} \sup T(t) \leq \frac{\gamma}{d_T}.
\]

**Proposition 8.** System (18) – (19) always has a tumor-free equilibrium \( E_0^* = (0, T_0^*) \) with \( T_0^* > 0 \). System (18) – (19) has a unique tumor-free equilibrium \( E_0^* = (0, T_0^*) \) with \( T_0^* > 0 \) if \( d_T > \lambda_{T_{12}} M \). System (18) – (19) has a tumorous equilibrium \( E_1^* = (V_1^*, T_1^*) \) if \( -d_T \alpha' \left( \frac{\gamma}{\eta} \right)^3 - (d_T - \lambda_{T_{12}} M) \left( \frac{\gamma}{\eta} \right) + \delta > 0 \).

**Proof.** If \((V^*, T^*)\) is an equilibrium of system (18)-(19), then we have \( V^*(r - \eta T^*) = 0 \), which implies that \( V^* = 0 \) or \( T^* = \frac{\gamma}{\eta} \). If \( V^* = 0 \) then \( \frac{\delta + \lambda_{T_{12}} T^* M}{1 + \alpha'} - d_T T^* = 0 \). Let \( p(T) = d_T \alpha' T^3 + (d_T - \lambda_{T_{12}} M) T - \delta \). Since \( p(0) = -\delta \) and \( \lim_{T \to \infty} p(T) = \infty \), we see that \( p(T) = 0 \) has at least one positive solution. Observe that \( p'(T) = 3d_T \alpha' T^2 + (d_T - \lambda_{T_{12}} M) > 0 \) if \( d_T - \lambda_{T_{12}} M > 0 \). Hence we conclude that if \( d_T > \lambda_{T_{12}} M \), then \( p(T) = 0 \) has a single positive root.

Next, when \( T^* = \frac{\gamma}{\eta} \), we see that

\[
\frac{1}{\frac{1}{1 + \alpha'} \left( T^* + \epsilon_v V^* \right)} - d_T T^* = 0,
\]

\[
d_T \alpha' T^3 + d_T \alpha' \epsilon_v V^* T^2 + (d_T - \lambda_{T_{12}} M) T^* - \delta = 0.
\]
The tumorous equilibrium
Proposition 10.

algebra manipulation/factoring for the $\lambda$ $\phi$ $\lambda$

The goal is to find some simple condition that ensures the eigenvalues are given by:

$\lambda$ $\phi$

The Jacobian for the tumor-free equilibrium
Proposition 9.

$\lambda$ $\phi$

To ensure the equilibrium is tumorous we need to ensure that $V^* > 0$, which implies

$\lambda$ $\phi$

Hence, a tumorous equilibrium is of the form

$E_1^* = (V_1^*, T_1^*) = \left( \frac{-d_T \alpha' \left( \frac{\xi}{\eta} \right)^3 - \left( d_T - \lambda_{T12} M \right) \left( \frac{\xi}{\eta} \right) + \delta}{d_T \alpha' \epsilon_\alpha \left( \frac{\xi}{\eta} \right)^2}, \frac{r}{\eta} \right)$.

The Jacobian for system (18) – (19) is given by:

$$
\begin{pmatrix}
    r - \eta T & -\eta V \\
    (\delta + \lambda_{T12} MT) \frac{\partial}{\partial T} F(V,T) & (\delta + \lambda_{T12} MT) \frac{\partial}{\partial T} F(V,T) + \lambda_{T12}MF(V,T) - d_T
\end{pmatrix}.
$$

Proposition 9. For the system (18)-(19) the following statements are true:

1. If $T_0^* > \max\{\frac{\xi}{\eta}, \sqrt{\frac{1}{\alpha}}\}$, then $E_0^*$ is a stable node.
2. If $\min\{\frac{\xi}{\eta}, \sqrt{\frac{1}{\alpha}}\} < T_0^* < \max\{\frac{\xi}{\eta}, \sqrt{\frac{1}{\alpha}}\}$, then $E_0^*$ is a saddle.
3. If $T_0^* < \min\{\frac{\xi}{\eta}, \sqrt{\frac{1}{\alpha}}\}$, then $E_0^*$ is an unstable node.

Proof. The Jacobian for the tumor-free equilibrium $E_0^* = (0, T_0^*)$ is the following:

$$
\begin{pmatrix}
    r - \eta T_0^* & 0 \\
    (\delta + \lambda_{T12} MT_0^*) \frac{\partial}{\partial T} F(0,T_0^*) & (\delta + \lambda_{T12} MT_0^*) \frac{\partial}{\partial T} F(0,T_0^*) + \lambda_{T12}MF(0,T_0^*) - d_T
\end{pmatrix}.
$$

As $F(V,T)$ is a decreasing function of $V$ and $T$, $\frac{\partial}{\partial T} F(V,T), \frac{\partial}{\partial V} F(V,T) < 0$, and the eigenvalues are given by:

$\lambda_1 = r - \eta T_0^* < 0$ if $T_0^* > \frac{r}{\eta}$,

$\lambda_2 = (\delta + \lambda_{T12} MT_0^*) \frac{\partial}{\partial T} F(0,T_0^*) + \lambda_{T12}MF(0,T_0^*) - d_T$.

The goal is to find some simple condition that ensures $\lambda_2 < 0$. We start by some algebra manipulation/factoring for the $\lambda_2$ expression.

$$
\lambda_2 = \delta \frac{\partial}{\partial T} F(0,T_0^*) - \frac{d_T}{2} + \lambda_{T12}M \left( T_0^* \frac{\partial}{\partial T} F(0,T_0^*) + F(0,T_0^*) \right).
$$

We seek to determine the sign for $\phi = T_0^* \frac{\partial}{\partial T} F(0,T_0^*) + F(0,T_0^*)$ which is equivalent to

$$
\phi = \frac{-\alpha'T_0^* + 1}{(1 + \alpha'T_0^*)^2}.
$$

Now, $\phi < 0$ if $-\alpha'T_0^* + 1 < 0$, which implies $T_0^* > \sqrt{\frac{1}{\alpha}}$ and $T_0^* < -\sqrt{\frac{1}{\alpha}}$ (unrealistic).

Next, we examine the local stability for the tumorous-equilibrium $E_1^* = (V_1^*, T_1^*)$.

Proposition 10. The tumorous equilibrium $E_1^* = (V_1^*, T_1^*)$ is a saddle point.
Proof. The Jacobian for the tumorous equilibrium is of the form:
\[
\begin{pmatrix}
0 & -\eta V^*_1 \\
W(T^*_1) \frac{\partial}{\partial V} F(V^*_1, T^*_1) & W(T^*_1) \frac{\partial}{\partial T} F(V^*_1, T^*_1) + \lambda_{T12} M F(V^*_1, T^*_1) - d_T
\end{pmatrix},
\]
where \(W(T^*_1) = \delta + \lambda_{T12} M T^*_1\). Its determinant is given by:
\[
\Delta = \eta V^*_1 (\delta + \lambda_{T12} M T^*_1) \frac{\partial}{\partial V} F(V^*_1, T^*_1) < 0.
\]
Since \(\Delta < 0\) the tumorous equilibrium is a saddle point.

We again use the Dulac criterion to obtain a condition that rules out the existence of positive periodic solutions for system (18) − (19).

**Proposition 11.** System \((18) − (19)\) has no nontrivial positive periodic solutions when \(T < \sqrt{\frac{1}{\alpha'}}\).

Proof. Let \(h(V, T) = \frac{1}{V}\). We can see:
\[
\Omega = \frac{\partial}{\partial V} \left\{ \frac{1}{V} (r V - \eta V T) \right\} + \frac{\partial}{\partial T} \left\{ \frac{1}{V} ((\delta + \lambda_{T12} M T) F(V, T) - d_T T) \right\}
\]
\[
= \frac{\partial}{\partial V} (r - \eta T) + \frac{\partial}{\partial T} F(V, T) + \lambda_{T12} M \frac{\partial}{\partial T} F(V, T) - d_T \frac{\partial}{\partial T} \frac{1}{V}
\]
\[
= \frac{\delta}{V} \frac{\partial}{\partial T} F(V, T) + \lambda_{T12} M \left( T \frac{\partial}{\partial T} F(V, T) + F(V, T) \right) - d_T \frac{\partial}{\partial T} \frac{1}{V}.
\]
We need to determine when \(\phi_1 = T \frac{\partial}{\partial T} F(V, T) + F(V, T) < 0\). A simple algebraic computation yields
\[
\phi_1 = \frac{-\alpha' T^2 + 1}{(1 + \alpha' T (T + \epsilon_V))^2}.
\]
Hence, \(\phi_1 < 0\) if \(T < \sqrt{\frac{1}{\alpha'}}\). Thus, the Dulac criterion ensures there will be no nontrivial positive periodic solution as long as \(T < \sqrt{\frac{1}{\alpha'}}\).

5. **Method for parameter estimation.** We estimate the parameters in the model based on (i) Literature review, (ii) Step-wise process by increasing model complexity with the application of a parameter estimation algorithm. All parameters values estimated by these ways are depicted on Table 2.

We first start with an extensive literature review to seek to estimate parameters for the model (3)-(6), as depicted in Section 9. For the remaining parameters, we numerically simulate the model using the MATLAB built-in function fmincon, which utilizes an interior point algorithm to minimize an objective error function within a range [3]. This way we are able to find the set of parameters that best fit the data in [29].

We calculate the error using the following formula:
\[
\hat{\theta} = \arg \min_{\{\theta \text{ feasible}\}} \sum_{i=1}^{N} (y^{\text{model}}(t_i; \theta) - y^{\text{observed}}_i)^2.
\]
Table 2. Parameters and variables (Var.) of the model system (3)-(6).

| Var.   | Meaning                                      | Value                  | Reference |
|--------|----------------------------------------------|------------------------|-----------|
| \(r\)  | Tumor cell growth rate                       | 0.213 day\(^{-1}\)     | fitted    |
| \(\eta\) | Kill rate of tumor cells by T cells             | 1 mm\(^{-3}\) day\(^{-1}\) | fitted    |
| \(\delta\) | Source of T cell activation                 | 0.02 mm\(^3\)/day      | estimated |
| \(\lambda_{T12}\) | Activation rate of T cells by IL-12        | 8.81 day\(^{-1}\)      | [14]      |
| \(K_{A2}\) | Dissociation constant of \(A_2\)          | 7 \times 10\(^{-14}\) moles/liter | estimated |
| \(K_{TQ}\) | Inhibition of function of T cells by PD-1-PD-L1 | 10\(^{-13}\) mm\(^6\) | estimated |
| \(d_T\) | Death rate of T cells                        | 0 – 0.5 day\(^{-1}\)   | [14]      |
| \(d_{A1}\) | Degradation rate of Anti-PD-L1              | 0.1136 day\(^{-1}\)   | [17]      |
| \(d_{A2}\) | Degradation rate of NHS-muIL12             | 0.69 day\(^{-1}\)     | [12]      |
| \(\rho_p\) | Expression level of PD-1                  | 3.19 \times 10\(^{-7}\) - 8.49 \times 10\(^{-7}\) | [11]      |
| \(\rho_L\) | Expression level of PD-L1                | 3.56 \times 10\(^{-7}\) - 1.967 \times 10\(^{-6}\) | [11]      |
| \(K_{A1}\) | Dissociation constant of free PD-L1 with anti-PD-L1 | 10\(^{-13}\) mol/liter | estimated |
| \(\epsilon_v\) | Expression of PD-L1 in tumor cells vs. T cells | 1-100                   | [14]      |
| \(\sigma\) | fraction of complex association and dissociation | 0.01 mm\(^{-3}\)   | estimated |
| \(\gamma_1\) | Continuous infusion rate of avelumab      | 10\(^{-7}\) – 9 \times 10\(^{-5}\) g/day | estimated |
| \(\gamma_2\) | Continuous infusion rate of NHS-muIL12   | 10\(^{-9}\) – 2 \times 10\(^{-6}\) g/day | estimated |
| \(c_1\) | Conversion constant for \(A_1\) drug        | 55\(^{-1}\)10\(^{-7}\) – 55\(^{-1}\)10\(^{-6}\) | estimated |
| \(c_2\) | Conversion constant for \(A_2\) drug        | 75\(^{-1}\)10\(^{-7}\) – 75\(^{-1}\)10\(^{-6}\) | estimated |

where \(N\) is the total number of data points, \(y_{model}\) is the solution of the tumor volume generated from the model under parameters \(\theta\) and evaluated at the corresponding data time points, and \(y_{observed}\) represents the tumor volume data points in [29]. We perform all simulations in MATLAB 2019a using the ODE system (3)-(6) and the following initial conditions: \(V(0) = 100, T(0) = 0.03, A_1(0) = 0\) and \(A_2(0) = 0\). The choice for the tumor volume initial condition is based on the mice initial tumor size \(\approx 100 mm^3\) [29].

6. Data fitting. We extract the data from Xu et al. [29] through the use of the application WebPlotDigitizer. In this experiment BALB/c mice were injected with EMT-6 breast cancer cells orthotopically into the mammary fat pad. Once the volume of the tumor reached approximately 100 mm\(^3\), the mice were randomly allocated into treatment groups consisting of eight mice each [29]. EMT-6 mice were treated both with monotherapy and combination treatments. The two types of treatments used were avelumab and NHS-muIL12. We consider the average tumor volume curves for each of the following cases: (i) Isotype control (200 \(\mu\)g), (ii) NHS-muIL12 (2 \(\mu\)g), (iii) NHS-muIL12 (10 \(\mu\)g), (iv) Avelumab (200 \(\mu\)g), (v) Avelumab (200 \(\mu\)g) + NHS-muIL12 (2 \(\mu\)g), (vi) Avelumab (200\(\mu\)g) + NHS-muIL12 (10\(\mu\)g).

We initially seek to determine which parameters are the main drivers of the tumor dynamics in each scenario. Two important parameters that affect the overall behavior of the system are the growth rate \((r)\) and the kill rate of the tumor cells.
by the T-cells ($\eta$). Using fmincon as described in Section 5 and trying a range of reasonable initial guesses, we fit the model to the no drug case data using the optimum guess and get the following estimates: $r = 0.2114$, $\eta = 1$. Note that all other parameters expressed in the no drug case are determined by literature review as shown on the parameter estimation table with $\gamma_1 = \gamma_2 = 0$. As depicted by Figure 2 a) below, the model accurately describes tumor growth in the absence of treatment.

We inherit these parameter estimates and sequentially fit the remaining parameters for the submodels using a similar approach as in [27]. For each submodel we determine the most sensitive parameters that affect the behavior of the system and fit those to data. This process eventually led to the fit of a higher in complexity model [27].

The first submodel captures the dynamics corresponding to treatment $A_2$ (NHS-muIL12). The drug $A_2$ was administrated subcutaneously on day 0 and dosages of 2 $\mu$g and 10 $\mu$g were explored [29]. We fit the parameter $K_{A_2}$ to NHS-muIL12 (2 $\mu$g) data and obtain the estimate $K_{A_2} = 7 \cdot 10^{-14}$, which differs from the estimate value in literature as shown in Section 9. Using the estimate for the parameter $K_{A_2}$, we fit for treatment NHS-muIL12 (10 $\mu$g). As observed on Figure 2 b) and c), the fit for case b) captures the behavior of the data qualitatively better than for case c).

We use a similar process for the $A_1$ (avelumab) data set. The drug $A_1$ was injected intravenously on days 0, 3 and 6 at a dosage level of 200 $\mu$g. Although based on our literature review $K_{A_1} = 1.8 \cdot 10^{-11}$ in Section 9, the model gives better fits when $K_{A_1} = 10^{-13}$ as depicted in Figure 2 d).

Now that we established appropriate parameter estimates for each case, we wish to examine whether the parameter estimates result into good predictions for the combination cases. We observe that the model predictions for the combination case avelumab (200 $\mu$g) and NHS-muIL12 (2 $\mu$g) as shown on Figure 2 e), are not satisfactory, implying that the model does not capture the eradication of the tumor as observed by the mice data. However, once the dosage of NHS-muIL12 increases to 10 $\mu$g, while maintaining the same dosage level for avelumab, the prediction tends to resemble closer the experimental data as observed in Figure 3.

7. Asymptotic behavior. We perform all simulations in MATLAB using the parameter estimates found during the data fitting process, whose values are listed in the table in Section 9. There are two asymptotic system behavior outcomes, namely tumor control and tumor escape. To distinguish between these two behaviors we define a threshold, $V_T$, such that if the solution computed at the last time point is greater than $V_T$, we define the outcome as “tumor escape”, otherwise as “tumor control.” We set $V_T = V(0)=100$, and use initial conditions $V(0) = 100, T(0) = 0.03, A_1(0) = 0$ and $A_2(0) = 0$. We consider the case when both drugs, i.e. NHS-muIL12 and avelumab, are applied continuously and run simulations for a long period of time ($\approx 220$ days) using both the full system (3)-(6) and the limiting system (18)-(19). We define the continuous dosages for avelumab and NHS-muIL12 as $\gamma_1$ and $\gamma_2$, respectively, and determine appropriate ranges from the data of Xu et al.[29]. Specifically, since avelumab is administered on days 0, 3, 6 over a time interval of 18 days and the dosage amount injected is 200$\mu$g, $\gamma_1$ results to $\gamma_1 \approx (3 \ast 200 \ast 10^{-6})/18 = 0.55 \ast 10^{-4} \frac{g}{\text{day}}$. Using a similar argument, $\gamma_2$ ranges between $\gamma_2 \approx 0.11 \ast 10^{-6} - 0.33 \ast 10^{-6} \frac{g}{\text{day}}$. 


Figure 2. Tumor volume data and simulations using model (3)-(6) for each single-agent and combination therapy case. Case (a) administration of no drug. Case (b), (c) treatment with NHS-muIL12 2 µg and 10 µg respectively on day 0. Case (d) administration of avelumab 200 µg on days 0, 3 and 6. Case (e) treatment with both avelumab (200 µg) on days 0, 3, 6 and NHS-muIL12 (2 µg) on day 0.

Based on this information, we create a surface plot (see Figure 4) where three regions are noted, namely, “tumor control”, “tumor escape” and “intermediate”. We are interested in seeing how the dynamics of the full system (3)-(6) compares to the dynamics of the limiting system (18)-(19). The intermediate region shown in Figure 4 underlines the difference between the dynamics of the two systems. Specifically, we select a point (red circle) within this region to demonstrate this behavior. Depending on which system is used the point lies either in the tumor escape or in the tumor control region. Overall the behavior of the two systems is qualitatively comparable. Hyperbolic curves separate the three regions. To achieve
Figure 3. First graph depicts the model simulation for the combination treatment case of avelumab (200 µg) and NHS-muIL12 (10 µg). The remaining two graphs correspond to each of the drugs administered with respect to time.

Figure 4. Surface plot depicting the asymptotic behavior of systems (3)-(6) and (18)-(19) for combinations of $\gamma_1$ (avelumab) and $\gamma_2$ (NHS-muIL12) drugs, where both are applied continuously. Based on a threshold, three regions, namely, ‘tumor control’, ‘tumor escape’ and ‘intermediate’ are identified, which are separated by hyperbolic curves. The hyperbolic curve closer to the origin corresponds to the system (18)-(19) and the other one to (3)-(6). Six representative points indicated by different markers and colors, are chosen and shown on the plot. Their respective tumor volume behavior is plotted in Figure 5 employing the same marker and color notation. Note that the red point falls in the intermediate region, where the behavior is dependent on the system being used.
smoother hyperbolic curves, we create a logarithmically spaced vector in MATLAB. We notice that there is synergism between the two drugs (avelumab and NHS-muIL12), meaning that the total effect of the drugs in combination is greater than the sum of administering the drugs individually. Particularly, we would require about one third of the amount of both drugs in combination to control the tumor in comparison to monotherapy.

8. Discussion. The last few years have seen a rapid increase in clinical trials investigating immune checkpoint inhibitors anti-PD-1/PD-L1 for a variety of types
of cancer. Although these agents have increased survival rates, not all patients respond to monotherapy treatment [30]. This has led to the examination of combination treatments that have complimentary effects [28]. Particularly, since 2018 there are approximately 2,250 active trials from which 1,760 are exploring the pairing of anti-PD-1/PD-L1 agents with other forms of treatments [26].

Xu et al. [29] concluded that combining anti-PD-L1 with NHS-muIL12 enhanced antitumor effectiveness in comparison to single agent treatment. Based on the data presented in their paper, we developed a simple biologically meaningful system of ordinary differential equations that models the tumor volume behavior. This work allowed us to complement and improve our previous work [14], where we only considered single agent treatments.

We performed mathematical analysis to a simple generic model (1)-(2) that allowed us to understand the behavior of the system. We established conditions, which we adapted to determine the dynamics for the exponential growth model. Moreover, we mathematically analyzed the continuous case of administrating both drugs (avelumab and NHS-muIL12) using the limiting system (18)-(19) in Section 4 and the full system (3)-(6) in Section 9. The mathematical analysis does not allow direct comparison of the dynamics of the systems, as it produces more technical results than biological insight. However, the mathematical analysis assists to the verification of the numerical predictions of the model.

Some of the parameters included in the model (3)-(6) proved to be challenging to calculate based on limited literature resources. However, the use of data in [29] allowed us to estimate biologically sensible ranges for those parameters based on submodel fitting. We were able to fit well the submodels of the full model to the monotherapy cases. Using these fit parameter values, model predictions indicate that we do not describe dual therapy at lower doses of NHS-muIL12 well. However, the model does make qualitatively good predictions at higher NHS-muIL12 doses. Given the reasonable predictions at higher NHS-muIL12 doses, we exploited the model to make predictions regarding treatment regimens not considered in the experiments of [29]. Specifically, we investigated the effects of administrating different combinations of avelumab and NHS-muIL12 continuously for both the full system (3)-(6) and the limiting system (18)-(19). We found that the dynamics of the systems are not equivalent, as system (18)-(19) is an approximation of (3)-(6). However, simulations suggested that the qualitative behavior of the two systems is comparable. Finally, we found that the drugs act synergistically and that approximately one third of the amount of both drugs could potentially eliminate the tumor.

Further investigation can be done in improving the structure of the model and determining whether we can find a model that yields a good fit to the monotherapy cases and both doses of NHS-muIL12. Additionally, for simplicity we determined parameters based on the average tumor volume of the eight mice. However, observing the individual tumor volume curves for each mouse reveals that the administration of treatments impacts each mouse in a different way. Hence, we could consider a more personalized approach by determining patient-specific parameters for each mouse. An example for such approach that considers patient specific parameters for GBM patients is noted in [6]. Furthermore, it would be interesting to explore how to potentially adapt the model to investigate the combination of different immune checkpoint inhibitors with other forms of treatment, such as chemotherapy.
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9. Appendix.

9.1. Parameter estimation. By performing a thorough literature review and additional calculations, we are able to estimate some of the parameters used in the model as follows:

Eq (3): The kill rate of tumor cells by T cells is estimated by [10] as $\eta = 1.101 \cdot 10^{-7}$ days$^{-1}$ cells$^{-1}$. We consider that on average cytotoxic T cells kill on a magnitude of 2-16 infected cells per day [5].

$$\eta = \frac{1.101 \cdot 10^{-7}}{\text{cells \cdot day}} \cdot 2 \text{ cells} - \frac{1.101 \cdot 10^{-7}}{\text{cells \cdot day}} \cdot 16 \text{ cells},$$

$$\eta = 2.202 \cdot 10^{-7} \text{ day}^{-1} - 17.616 \cdot 10^{-7} \text{ day}^{-1}.$$

Eq (4): According to [15] the dissociation constant for IL-12 is $0.018 \text{ pmol \cdot mL}^{-1} \approx 1.8 \cdot 10^{-11} \text{ M}$. Since the dissociation constant is terms of M (molarity) we need to make sure that the units are consistent. We observe the following units for the $dT/dt$ equation:

$$\frac{dT}{dt} = \left( \delta + \frac{\lambda T I_{12}}{m m^3} \cdot \frac{A'_2}{m m^3} \cdot \frac{A_2}{m m^3} ight) \cdot \frac{1}{1 + \frac{Q}{m m^3}} \cdot \frac{d_T}{m m^3}.$$

Observing more closely the term:

$$\frac{A'_2}{m m^3} \cdot \frac{A_2}{m m^3} = \frac{A'_2}{K_{A_2} + A'_2} \text{ where } A'_2 = \frac{A_2 \text{ molar mass}}{\text{tumor volume}}.$$

We note that $A_2$ is originally given in g and the goal is to convert to molarity ($\text{mol \cdot L}^{-1}$). We first convert to moles as follows:

$$A_2(g) = \frac{A_2}{\text{molar mass}(\text{g \cdot mol})}.$$

According to Sigma-Aldrich source the molar mass of IL-12 is 75KDa, which is approximately 75,000 grams per mole. Retrieved from: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Datasheet/2/i2276dat.pdf.

$$\frac{A_2(g)}{75,000(\text{g \cdot mol})} = \frac{A_2}{75,000} \text{ mol}.$$

Given moles we can now convert to molarity which we denote by $c$:

$$c = \frac{n}{v} \text{ where } n = \# \text{ of moles and } v = \text{volume in (liters)},$$
Regarding $v$ (tumor volume in liters), one can observe from Figure 1A that the tumor volume ranges between $(100 - 1000) \text{ mm}^3 \approx (10^{-4} - 10^{-3}) \text{ l}$ depending on the type of treatment administrated. For simplicity we will assume that the whole portion of the drug targets the tumor volume.

\[ c_2 = \frac{A_2}{v} = \frac{75,000}{v}. \]

Eq (5): According to the American College of Clinical Pharmacology (ACCP) and [17], the half life of avelumab is 6.1 days. Hence, the degradation rate constant ($d_{A_1}$) can be calculated as follows:

\[ d_{A_1} = \frac{\ln 2}{t_{\frac{1}{2}}} = \frac{\ln 2}{6.1} \approx 0.1136 \text{ days}^{-1}. \]

Eq (6): According to [12] the circulatory half-life of NHS-IL12 in vivo is approximately 24 hours. Hence, the degradation rate constant ($d_{A_2}$) can be calculated as follows:

\[ d_{A_2} = \frac{\ln 2}{t_{\frac{1}{2}}} = \frac{\ln 2}{1} \approx 0.69 \text{ days}^{-1}. \]

9.2. Mathematical analysis for the continuous treatment case: Full system. The full system consists of four nonlinear equations where $\gamma_1$ and $\gamma_2$ represent the continuous injection of NHS-muIL12 and Avelumab respectively.

\[ \frac{dV}{dt} = rV - \eta VT, \quad (23) \]

\[ \frac{dT}{dt} = \left( \delta + \lambda_T T - \frac{c_2 A_2}{K_{A_2} + c_2 A_2} \right) \cdot \frac{1}{1 + \frac{Q(V,T,A_1)}{K_{TQ}}} - d_T T, \quad (24) \]

\[ \frac{dA_1}{dt} = \gamma_1 - d_{A_1} A_1, \quad (25) \]

\[ \frac{dA_2}{dt} = \gamma_2 - d_{A_2} A_2. \quad (26) \]
Table 3. Parameter values used in simulations for equations (3)-(6).

| Variable | Meaning | Value |
|----------|---------|-------|
| r        | Tumor cell growth rate | 0.213 day$^{-1}$ |
| $\eta$   | Kill rate of tumor cells by T cells | 1 mm$^{-3}$ day$^{-1}$ |
| $\delta$ | Source of activation | 0.02 mm$^3$ day$^{-1}$ |
| $\lambda_{T_{12}}$ | Activation rate of T cells by IL-12 | 8.81 day$^{-1}$ |
| $K_{A_2}$ | Dissociation constant of $A_2$ | $7 \cdot 10^{-14}$ moles/liter |
| $K_{TQ}$ | Inhibition of function of T cells by PD-1-PD-L1 | $10^{-13}$ mm$^6$ |
| $d_T$    | Death rate of T cells | 0.05 day$^{-1}$ |
| $d_{A_1}$ | Degradation rate of Anti-PD-L1 | 0.1136 day$^{-1}$ |
| $d_{A_2}$ | Degradation rate of NHS-muIL12 | 0.69 day$^{-1}$ |
| $\rho_p$ | Expression level of PD-1 | 5.84 $\cdot 10^{-7}$ |
| $\rho_L$ | Expression level of PD-L1 | 2.7635 $\cdot 10^{-7}$ |
| $K_{A_1}$ | Dissociation constant of PD-L1 with anti-PD-L1 | $10^{-13}$ mol/liter |
| $\epsilon_v$ | Expression of PD-L1 in tumor cells vs. T cells | 50 |
| $\sigma$ | fraction of complex association and dissociation | 0.001 mm$^{-3}$ |
| $\gamma_1$ | prescriptive infusion rate of avelumab | $10^{-7} - 9 \cdot 10^{-5}$ g/day |
| $\gamma_2$ | prescriptive infusion rate of NHS-muIL12 | $10^{-9} - 2 \cdot 10^{-6}$ g/day |
| $c_1$ | conversion constant for $A_1$ drug | $55 - 10^{-7}$ |
| $c_2$ | conversion constant for $A_2$ drug | $75 - 10^{-7}$ |

where,

$$F(V, T, A_1) = \frac{1}{1 + \frac{Q(V, T, A_1)}{K_{TQ}}}$$

$$= \frac{1}{1 + \alpha' T (T + \epsilon_v V)(1 - \frac{c_1 A_1}{c_1 A_1 + K_{A_1}})}$$

with,

$$\alpha' = \frac{\sigma \rho_p \rho_L}{K_{TQ}}.$$ 

It is easy to see that the solution of the above system (23) – (26) with positive initial values is positive. We use a similar argument as when performing mathematical analysis for the limiting system (18)-(19), and conclude that boundedness for $V$ cannot be established. However, the following proposition is true.

**Proposition 12.** The $T, A_1$ and $A_2$ components of the solutions of the equations (23) – (26) are bounded.

**Proof.** It is easy to see that solutions for both $A_1$ and $A_2$ are bounded. Additionally, for positive values of $V$ and $T$ and $A_1$ the function $\left(\delta + \lambda_{T_{12}} T \frac{c_2 A_2}{K_{A_2} + c_2 A_2}\right) F(V, T, A_1) < \gamma$ for some positive constant $\gamma$. This implies

$$\frac{dT}{dt} < \gamma - d_T T.$$ 

By comparison argument, we see that $T \leq \max\{T_0, \frac{\gamma}{d_T}\}$ and

$$\lim_{t \to \infty} \sup_{t} T(t) \leq \frac{\gamma}{d_T}.$$ 

$\Box$
Proposition 13. System (23) – (26) has at least a tumor-free equilibrium $E_0^* = (0, T_0, \frac{\gamma_1}{d_{A_1}}, \frac{\gamma_2}{d_{A_2}})$ with $T_0^* > 0$. System (23) – (26) has a unique tumor-free equilibrium $E_0^* = (0, T_0^*, \frac{\gamma_1}{d_{A_1}}, \frac{\gamma_2}{d_{A_2}})$ with $T_0^* > 0$ if $(d_T - \lambda_T T_12 \frac{\gamma_1}{d_{A_1}} \frac{\gamma_2}{d_{A_2}}) > 0$.

Proof. We set $\frac{dV}{dt} = 0$, which implies that $V^* = 0$ and $T^* = \frac{\gamma_1}{\eta}$. We set $\frac{dA_1}{dt} = 0$ and get $A_1^* = \frac{\gamma_1}{d_{A_1}}$ and $A_2^* = \frac{\gamma_2}{d_{A_2}}$ respectively.

If $V^* = 0$, $A_1^* = \frac{\gamma_1}{d_{A_1}}$ and $A_2^* = \frac{\gamma_2}{d_{A_2}}$ then

$$\left(\delta + \lambda_T T_12 T^* \frac{c_2 \gamma_2}{d_{A_2} K_{A_2} + c_2 \gamma_2} \right) \cdot \frac{1}{1 + \alpha T^* (1 - \frac{c_1 \gamma_1}{c_2 \gamma_1 + c_2 \gamma_2})} - d_T T^* = 0. \quad (27)$$

Let $p(T^*) = d_T \alpha' (1 - \frac{c_1 \gamma_1}{c_2 \gamma_1 + c_2 \gamma_2}) T^* + (d_T - \lambda_T T_12 \frac{c_2 \gamma_2}{d_{A_2} K_{A_2} + c_2 \gamma_2}) T^* - \delta$. We see that $p(T^*) = 0$ if and only if equation (27) holds. Since $p(0) = -\delta$ and $\lim_{T \to \infty} p(T) = \infty$, we see that $p(T) = 0$ has at least one positive solution. Observe that $1 - \frac{c_1 \gamma_1}{c_2 \gamma_1 + c_2 \gamma_2} > 0$ and $p'(T^*) = 3d_T \alpha T^* (1 - \frac{c_1 \gamma_1}{c_2 \gamma_1 + c_2 \gamma_2}) + (d_T - \lambda_T T_12 \frac{c_2 \gamma_2}{d_{A_2} K_{A_2} + c_2 \gamma_2})$. We see that $p'(T^*) > 0$, provided $(d_T - \lambda_T T_12 \frac{c_2 \gamma_2}{d_{A_2} K_{A_2} + c_2 \gamma_2}) > 0$, in which case, $p(T)$ has a single positive root.

In the following, we let

$$\Delta(T, A_2) = \delta + \lambda_T T_12 T \frac{c_2 A_2}{K_{A_2} + c_2 A_2}.$$ 

The following proposition presents a simple necessary and sufficient condition for system (23) – (26) to have a unique tumorous equilibrium.

Proposition 14. Let $D = \Delta(r/\eta, \gamma_2/d_{A_2})$. System (23) – (26) has a unique tumorous equilibrium $E^* = (V^*, T^*, A_1^*, A_2^*)$ if and only if

$$\frac{D \eta}{rd_T} > 1 + Q(0, \frac{r}{\eta}, \frac{\gamma_1}{d_{A_1}}).$$

Proof. Observe that if the system (23) – (26) has a tumorous equilibrium $E^* = (V^*, T^*, A_1^*, A_2^*)$, then $T^* = \frac{\gamma_1}{\eta}$, $A_1^* = \frac{\gamma_1}{d_{A_1}}$ and $A_2^* = \frac{\gamma_2}{d_{A_2}}$. Straightforward computation from equation (24) yields

$$\frac{D \eta}{rd_T} = 1 + Q(V^*, \frac{r}{\eta}, \frac{\gamma_1}{d_{A_1}}) \equiv G(V^*).$$

Observe that $G(V^*)$ is a strictly increasing function for $V^* \geq 0$. This implies that if

$$\frac{D \eta}{rd_T} > 1 + Q(0, \frac{r}{\eta}, \frac{\gamma_1}{d_{A_1}}),$$

then

$$G(V^*) > 1 + Q(0, \frac{r}{\eta}, \frac{\gamma_1}{d_{A_1}}) = G(0),$$

which implies that $V^* > 0$. Since $G(V^*)$ is strictly monotone, we see that in such case, the solution $V^*$ of $G(V^*) = \frac{D \eta}{rd_T}$ is unique.

The Jacobian for the system (23)-(26) is given by:

$$\begin{pmatrix}
r - \eta T & -\eta V & 0 & 0 \\
a_{21} & a_{22} & a_{23} & a_{24} \\
0 & 0 & -d_{A_1} & 0 \\
0 & 0 & 0 & -d_{A_2}
\end{pmatrix}$$
where
\[
\begin{align*}
  a_{21} &= \Delta(T, A_2) \frac{\partial F(V, T, A_1)}{\partial V}, \\
  a_{22} &= \left( \lambda_{TT_1} \frac{c_2 K_{A_2}}{K_{A_2} + c_2 A_2} \right) F(V, T, A_1) + \Delta(T, A_2) \frac{\partial F(V, T, A_1)}{\partial T} - d_T, \\
  a_{23} &= \Delta(T, A_2) \frac{\partial F(V, T, A_1)}{\partial A_1}, \\
  a_{24} &= F(V, T, A_1) \lambda_{TT_1} T \frac{c_2 K_{A_2}}{(K_{A_2} + c_2 A_2)^2}.
\end{align*}
\]

**Proposition 15.** The system (23) – (26) has a stable tumor-free equilibrium provided that \(T_0^* > \frac{r}{\eta}\) and \(F(0, T_0^*, \frac{\gamma_1}{d_{A_1}}, \frac{\gamma_1}{d_{A_1}}) < 0\).

**Proof.** The Jacobian for the tumor-free equilibrium \(E_0^* = (0, T_0^*, \frac{\gamma_1}{d_{A_1}}, \frac{\gamma_1}{d_{A_1}})\) is given by:

\[
\begin{pmatrix}
  r - \eta T_0^* & 0 & 0 & 0 \\
  a_{21} & a_{22} & a_{23} & a_{24} \\
  0 & 0 & -d_{A_1} & 0 \\
  0 & 0 & 0 & -d_{A_2}
\end{pmatrix}
\]

where
\[
\begin{align*}
  a_{21} &= \Delta(T_0^*, \frac{\gamma_1}{d_{A_1}}) \frac{\partial F(0, T_0^*, \frac{\gamma_1}{d_{A_1}})}{\partial V}, \\
  a_{22} &= \left( \lambda_{TT_1} \frac{c_2 K_{A_2}}{K_{A_2} + c_2 A_2} \right) F(0, T_0^*, \frac{\gamma_1}{d_{A_1}}) + \Delta(T_0^*, \frac{\gamma_1}{d_{A_1}}) \frac{\partial F(0, T_0^*, \frac{\gamma_1}{d_{A_1}})}{\partial T} - d_T, \\
  a_{23} &= \Delta(T_0^*, \frac{\gamma_1}{d_{A_1}}) \frac{\partial F(0, T_0^*, \frac{\gamma_1}{d_{A_1}})}{\partial A_1}, \\
  a_{24} &= F(0, T_0^*, \frac{\gamma_1}{d_{A_1}}) \lambda_{TT_1} T \frac{c_2 K_{A_2}}{(K_{A_2} + c_2 A_2)^2}.
\end{align*}
\]

As \(F(V, T, A_1)\) is a decreasing function of \(V, T\) and \(A_1, \frac{\partial}{\partial V} F(V, T, A_1), \frac{\partial}{\partial T} F(V, T, A_1), \frac{\partial}{\partial A_1} F(V, T, A_1) < 0\), and the eigenvalues are given by:

\[
\begin{align*}
  \lambda_1 &= r - \eta T_0^* < 0 \text{ if } T_0^* > \frac{r}{\eta}, \\
  \lambda_2 &= a_{22}, \\
  \lambda_3 &= -d_{A_1} < 0, \\
  \lambda_4 &= -d_{A_2} < 0.
\end{align*}
\]

Regarding the sign of \(a_{22}, a_{22}\) can be either positive or negative.

\[
\begin{align*}
  a_{22} &= \left( \lambda_{TT_1} \frac{c_2 K_{A_2}}{K_{A_2} + c_2 A_2} \right) F(0, T_0^*, \frac{\gamma_1}{d_{A_1}}) + \Delta(T_0^*, \frac{\gamma_1}{d_{A_1}}) \frac{\partial F(0, T_0^*, \frac{\gamma_1}{d_{A_1}})}{\partial T} - d_T.
\end{align*}
\]

If \(F(0, T_0^*, \frac{\gamma_1}{d_{A_1}}) < 0\), then \(a_{22} < 0\), which implies that \(E_0^*\) is stable. \(\square\)
9.3. **Supplementary figures.** The following figures support the generation of Figure 4.

**Figure 6.** Surface plot depicting the asymptotic behavior of the full system (3)-(6) for combinations of $\gamma_1$ (avelumab) and $\gamma_2$ (NHS-muIL12) drugs, where both are applied continuously. Based on a threshold, two regions, namely, ‘Tumor Control’ and ‘Tumor Escape’ are identified, which are separated by a hyperbolic curve. Six representative points indicated by different markers and colors, are chosen and shown on the plot.

**Figure 7.** Surface plot depicting the asymptotic behavior of the limit case system (18)-(19) for combinations of $\gamma_1$ (avelumab) and $\gamma_2$ (NHS-muIL12) drugs, where both are applied continuously. Based on a threshold, two regions, namely, ‘Tumor Control’ and ‘Tumor Escape’ are identified, which are separated by a hyperbolic curve. Six representative points indicated by different markers and colors, are chosen and shown on the plot.
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