Introduction

When cancer cells undergo necrosis, they release high mobility group box-1 (HMGB-1) which activates dendritic cells [1–3]. Activated dendritic cells (DCs) mature as APC cells and play a critical role in the communication between the innate and adaptive immune responses. Once activated, dendritic cells produce IL-12, which activates effector T cells CD4+ Th1 and CD8+ T [4, 5]. Th1 produces IL-2 which further promotes proliferation of the effector T cells. Both CD4+ Th1 and CD8+ T cells kill cancer cells [6–8]. CD8+ T cells are more effective in killing cancer cells, but the helper function of CD4+ Th1 cells improves the efficacy of tumor-reactive CD8+ T cells [9].
Cancer vaccines serve to enlarge the pool of tumor-specific T cells from the naive repertoire, and also to activate tumor specific T cells which are dormant [10]. GM-CSF can activate dendritic cells, and is commonly used as a cancer vaccine [11–13]. GVAX is a cancer vaccine composed of tumor cells genetically modified to secrete GM-CSF and then irradiated to prevent further cell division.

PD-1 is an immunoinhibitory receptor predominantly expressed on activated T cells [14, 15]. Its ligand PD-L1 is upregulated on the same activated T cells, but it is also expressed by some human cancer cells, such as in melanoma, lung cancer, colon cancer, and leukemia [15–17]. The complex PD-1-PD-L1 is known to inhibit T cell function [14]. Immune checkpoints are regulatory pathways in the immune system that inhibit its active response against specific targets. In the case of cancer, the complex PD-1-PD-L1 forms an immune checkpoint for T cells.

There has been much progress in recent years in developing checkpoint inhibitors, primarily PD-1 antibodies and PD-L1 antibodies [17]. Such drugs have been increasingly explored in single-agent studies for cancer treatment [16, 18]. The FDA recently approved several checkpoint inhibitors. However, because of lack of tumor-infiltrating effector T cells, many patients in clinical trials do not respond to checkpoint inhibitor treatment [18]. On the other hand, cancer vaccines have been shown to induce effector T-cells infiltration into tumors [19], although, to be fully effective, cancer vaccines have to overcome immune evasion [10]. It was recently suggested that the combination of a cancer vaccine and an immune checkpoint inhibitor may function synergistically to induce more effective antitumor immune responses [18, 20]. Clinical trials to test such combination therapies are currently ongoing [18, 20]; mouse experiments are also being conducted [21–27].

In the present paper we develop a mathematical model of treatment of cancer with a cancer vaccine combined with an immune checkpoint inhibitor; specifically, we combine GVAX and PD-1 inhibitor. In order to focus on the combination therapy of the two drugs, we consider in the model only the following variables: cancer cells (C), dendritic cells (DCs), CD4$^+$ and CD8$^+$ T cells, GM-CSF, PD-1, PD-L1, PD-1-PD-L1 complex, and cytokines IL-12 and IL-2. These species interact within the network shown in Fig 1. The mathematical model is based on Fig 1, and it is represented by a system of partial differential equations (PDEs). Simulations of the model are shown to be in qualitative agreement with the mouse experiments reported in [21–23]. The model is then used to explore the efficacy of the combined treatment. We introduce a specific concept of synergy between the vaccine and the PD-1 inhibitor, which is somewhat different from the usual definition of synergy. Roughly speaking, we compare the reduction in tumor size achieved by a combined therapy with amounts $\gamma_G$ of GVAX and $\gamma_A$ of PD-1 inhibitor to the reduction obtained by single-agent with either $(1 + \theta_G)\gamma_G$ or $(1 + \theta_A)\gamma_A$ with appropriately chosen $0 < \theta_G \leq 1$ or $0 < \theta_A \leq 1$. The larger the reduction in tumor size achieved by the combination therapy the larger the synergy is said to be. A specific choice of $\theta_G$ and $\theta_A$, which takes into account potential negative side-effects of each drug, will be given as an example. We develop a synergy map in the $(\gamma_G, \gamma_A)$-plane. The map shows that, given $\gamma_G$, the synergy increases as $\gamma_A$ increases as long as $\gamma_A$ remains below a critical value $\gamma_{AG}$; thereafter, the synergy decreases as $\gamma_A$ increases.

A discrete-time mathematical model of the combination of radiotherapy with checkpoint inhibitors was developed in [28]. Mathematical models of immunotherapy with a cancer vaccine by a system of ordinary differential equations were developed earlier in [29, 30]; these models do not consider checkpoint inhibitors. In [29] it is shown that there is a positive correlation between the number of antigen presenting cells and prolonged cancer dormancy, and in [30] it is illustrated how the combined therapy can eliminate the cancer, though neither immunotherapy nor checkpoint inhibitors can do it alone. In this paper, we present for the first time
a mathematical model which combines a cancer vaccine with a checkpoint inhibitor: the vaccine (GVAX) increases the pool of T cells and the checkpoint inhibitor (anti-PD-1) enables the T cells to remain fully active in killing cancer cells.

**Mathematical model**

The mathematical model is based on the network shown in Fig 1. The list of variables, with units, is given in Table 1.

**Table 1. List of variables.**

| Notation | Description                        | units   |
|----------|------------------------------------|---------|
| D        | density of DCs                     | g/cm³   |
| T₁       | density of activated CD4⁺ T cells  | g/cm³   |
| T₂       | density of activated CD8⁺ T cells  | g/cm³   |
| C        | density of cancer cells            | g/cm³   |
| N_C      | density of necrotic cancer cells   | g/cm³   |
| H        | HMG-1 concentration                | g/cm³   |
| G        | GM-CSF concentration               | g/cm³   |
| h₁₂      | IL-12 concentration                | g/cm³   |
| b₂       | IL-2 concentration                 | g/cm³   |
| P        | PD-1 concentration                 | g/cm³   |
| L        | PD-L1 concentration                | g/cm³   |
| Q        | PD-1-PD-L1 concentration           | g/cm³   |
| A        | anti-PD-1 concentration            | g/cm³   |

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We assume that the total density of cancer cells \((C)\), active dendritic cells \((D)\), CD4\(^+\) T cells \((T_i)\) and CD8\(^+\) T cells \((T_s)\) within the tumor remains constant in space and time:

\[
C + D + T_i + T_s = \text{constant.} \tag{1}
\]

It is tacitly assumed that the debris of dead cells, including cancer cells undergoing necrosis or apoptosis, is quickly cleared from the tumor tissue. It is also tacitly assumed that the densities of immature dendritic cells and naive CD4\(^+\) and CD8\(^+\) T cells are constant throughout the tumor tissue.

Under the assumption Eq (1), cancer cell proliferation, and migration of immune cells into the tumor give rise to internal pressure which results in cell movement, and we assume that all the cells move with the same velocity, \(u\); \(u\) depends on space and time. We also assume that all the cells undergo diffusion, and that all the cytokines and drugs are diffusing within the tumor.

**Equation for DCs (\(D\)).** When cancer cells undergo necrosis, they release HMGB-1 [1]. We can model the dynamics of the necrotic cells and of HMGB-1 by the following equations:

\[
\frac{\partial N_C}{\partial t} + \nabla \cdot (uN_C) - \delta_{NC} \nabla^2 N_C = \lambda_{NC}C - d_{NC}N_C,
\]

\[
\frac{\partial H}{\partial t} - \delta_{H} \nabla^2 H = \lambda_{HNC} N_C - d_{H}H,
\]

where \(\lambda_{NC}\) is the rate at which cancer cells become necrotic and \(\lambda_{HNC}\) is the rate at which necrotic cells produce HMGB-1. We note that although molecules like HMGB-1, or other proteins, may be affected by the velocity \(u\), their diffusion coefficients are several orders of magnitude larger than the diffusion coefficients of cells; hence their velocity terms may be neglected. The degradation of HMGB-1 is fast \((\sim 0.01/\text{day})\) [31], and we assume that the removal of \(N_C\) is also fast. We can therefore approximate the two dynamical equations by the steady state equations \(\lambda_{NC}C - d_{NC}N_C = 0\) and \(\lambda_{HNC}N_C - d_{H}H = 0\), so that \(H\) is proportional to \(C\), i.e., \(H = \text{constant} \times C\).

Dendritic cells are activated by HMGB-1 [2, 3]. Hence, the activation rate of immature dendritic cell \(D_0\) is proportional to \(D_0 \frac{C}{K_C + C}\), where the Michaelis-Menten law is used to account for the limited rate of receptor recycling time which occurs in the process of DCs activation. In the same way, GM-CSF, produced by the cancer vaccine, activates DCs at rate proportional to \(D_0 \frac{G}{K_G + G}\). Hence, the dynamics of DCs is given by

\[
\frac{\partial D}{\partial t} + \nabla \cdot (uD) - \delta_D \nabla^2 D = \lambda_{DC}D_0 \frac{C}{K_C + C} + \lambda_{DG}D_0 \frac{G}{K_G + G} - d_D D,
\]

where \(\delta_D\) is the diffusion coefficient and \(d_D\) is the death rate of DCs.

**Equation for CD4\(^+\) T cells (\(T_i\)).** Naive CD4\(^+\) T cells are activated by IL-12, and IL-2 induces proliferation of activated \(T_i\) cells [4, 5]. Both processes are assumed to be inhibited by the complex PD-1-PD-L1 \((Q)\) [14], which reduces the production of \(T_i\) cells by a factor \(\frac{1}{1+Q/K_Q}\).
Hence $T_t$ satisfies the following equation:

$$
\frac{\partial T_t}{\partial t} + \nabla \cdot (u T_t) - \delta T \nabla^2 T_t = \left( \frac{\lambda_{T,t_{12}} T_{t_{12}} I_{12}}{K_{t_{12}} + I_{12}} + \frac{\lambda_{T,t_{12}} T_{t_{12}} I_{2}}{K_{t_{12}} + I_{2}} \right)_{\text{activation by IL-12}}

\times \frac{1}{1 + Q/K_{TQ}} - d_T T_t,_{\text {death}}
$$

where $T_{t_{12}}$ is the density of naive CD4$^+$ T cells.

**Equation for activated CD8$^+$ T cells ($T_8$).** IL-12 activates CD8$^+$ T cells and IL-2 induces the proliferation of CD8$^+$ T cells [4, 5]. Hence, similarly to the equation for $T_t$, $T_8$ satisfies the equation

$$
\frac{\partial T_8}{\partial t} + \nabla \cdot (u T_8) - \delta T \nabla^2 T_8 = \left( \frac{\lambda_{T,t_{12}} T_{t_{12}} I_{12}}{K_{t_{12}} + I_{12}} + \frac{\lambda_{T,t_{12}} T_{t_{12}} I_{2}}{K_{t_{12}} + I_{2}} \right)_{\text{activation by IL-12}}

\times \frac{1}{1 + Q/K_{TQ}} - d_T T_8,_{\text {death}}
$$

where $T_{t_{12}}$ is the density of naive CD8$^+$ T cells.

**Equation for tumor cells ($C$).** Cancer cells are killed by $T_t$ and $T_8$ [6–8]. We assume a logistic growth with carrying capacity ($C_M$) in order to account for competition for space among the cancer cells. Hence,

$$
\frac{\partial C}{\partial t} + \nabla \cdot (u C) - \delta C \nabla^2 C = \lambda_C C \left( 1 - \frac{C}{C_M} \right)_{\text{proliferation}} - \left( \eta_1 T_t C + \eta_8 T_8 C \right)_{\text{killing by T cells}} - d_C C,_{\text {death}}
$$

where $\eta_1$, $\eta_8$ are the killing rates of cancer cells by $T_t$ and $T_8$, and $d_C$ is the natural death rate of cancer cells.

**Equation for GM-CSF ($G$).** We assume that GVAX is injected intradermally every 3 days for 30 days (as in mouse experiments [21]) providing a source $\hat{G}(t)$ of GM-CSF, which we represent by

$$
\hat{G}(t) = \begin{cases} 
\gamma_G & \text{if } t \leq 30, \\
\gamma_G \times \frac{3(t-30)}{90} & \text{if } 30 < t \leq 33, \\
0 & \text{if } t > 33.
\end{cases}
$$

where $\gamma_G$ is the effective level of the drug; although the level of the drug varies between injections, for simplicity we take it to be constant. The concentration of GM-CSF in tissue is very small [32], and accordingly, we assume a low rate of constant source $\lambda_G$ for GM-CSF. Hence $G$ satisfies the following equation:

$$
\frac{\partial G}{\partial t} - \delta_G \nabla^2 G = \lambda_G + \hat{G}(t) - d_G G,_{\text {degradation}}
$$

where $d_G$ is the degradation rate of GM-CSF.
Equation for IL-12 ($I_{12}$). IL-12 is produced by activated DCs [4, 5], so that

$$\frac{\partial I_{12}}{\partial t} - \delta_{I_{12}} \nabla^2 I_{12} = \frac{\lambda_{I_{12}D}}{\text{production by DCs}} - d_{I_{12}} I_{12}.$$  \hspace{1cm} (7)

Equation for IL-2 ($I_2$). IL-2 is produced by activated CD4$^+$ T cells ($T_1$) [4, 5]. Hence,

$$\frac{\partial I_2}{\partial t} - \delta_{I_2} \nabla^2 I_2 = \frac{\lambda_{I_2 T_1}}{\text{production by } T_1} - d_{I_2} I_2.$$  \hspace{1cm} (8)

Equation for PD-1 ($P$), PD-L1 ($L$) and PD-1-PD-L1 ($Q$). PD-1 is expressed on the surface of activated CD4$^+$ T cells and activated CD8$^+$ T cells [14, 15]. We assume that the expression level of PD-1 is the same for activated CD4$^+$ and CD8$^+$ T cells. Hence, $P$ is given by

$$P = \rho_P (T_1 + T_8),$$

where $\rho_p$ is the ratio between the mass of one PD-1 protein to the mass of one T cell. The coefficient $\rho_p$ is constant when no anti-PD-1 drug is injected. In that case, to a change in $T = T_1 + T_8$, given by $\frac{\partial P}{\partial t}$, there corresponds a change of $P$, given by $\rho_P \frac{\partial P}{\partial t}$. For the same reason, $\nabla \cdot (uP) = \rho_P \nabla \cdot (uT)$ and $\nabla^2 P = \rho_P \nabla^2 T$ when no anti-PD-1 drug is injected. Hence, $P$ satisfies the equation

$$\frac{\partial P}{\partial t} + \nabla \cdot (uP) - \delta_T \nabla^2 P = \rho_P \left[ \frac{\partial(T_1 + T_8)}{\partial t} + \nabla \cdot (u(T_1 + T_8)) - \delta_T \nabla^2 (T_1 + T_8) \right].$$

Recalling Eqs (3) and (4) for $T_1$ and $T_8$, we get

$$\frac{\partial P}{\partial t} + \nabla \cdot (uP) - \delta_T \nabla^2 P = \rho_P \left[ \frac{\lambda_{T_1 T_{10}}}{K_{T_{10}}} + \frac{\lambda_{T_1 T_{30}}}{K_{T_{30}}} \right] \frac{I_{12}}{K_{I_{12}} + I_{12}} + \frac{\lambda_{T_8 T_{10}}}{K_{T_{10}}} \frac{I_{2}}{K_{I_{2}} + I_{2}} \times \frac{1}{1 + Q/K_{TQ}} - \rho_P (d_{T_1} T_1 + d_{T_8} T_8).$$

When anti-PD-1 drug ($A$) is applied, PD-1 is depleted (or blocked) by $A$. In this case, the ratio $\frac{\rho_p}{T_1 + T_8}$ may change. In order to include in the model both cases of with and without anti-PD-1, we replace $\rho_p$ in the previous equation by $\frac{\rho_p}{T_1 + T_8}$. Hence,

$$\frac{\partial P}{\partial t} + \nabla \cdot (uP) - \delta_T \nabla^2 P = \frac{P}{T_1 + T_8} \left[ \frac{\lambda_{T_1 T_{10}}}{K_{T_{10}}} + \frac{\lambda_{T_1 T_{30}}}{K_{T_{30}}} \right] \frac{I_{12}}{K_{I_{12}} + I_{12}} + \frac{\lambda_{T_8 T_{10}}}{K_{T_{10}}} \frac{I_{2}}{K_{I_{2}} + I_{2}} \times \frac{1}{1 + Q/K_{TQ}} - \rho_p (d_{T_1} T_1 + d_{T_8} T_8) - \frac{\mu_{PA}}{T_1 + T_8} (d_{T_1} T_1 + d_{T_8} T_8)$$

$$\hspace{1cm} = \frac{P}{T_1 + T_8} \left[ (\lambda_{T_1 T_{10}} + \lambda_{T_1 T_{30}}) \frac{I_{12}}{K_{I_{12}} + I_{12}} + (\lambda_{T_8 T_{10}} + \lambda_{T_8 T_{30}}) \frac{I_{2}}{K_{I_{2}} + I_{2}} \right] \times \frac{1}{1 + Q/K_{TQ}} - \rho_p (d_{T_1} T_1 + d_{T_8} T_8) - \frac{\mu_{PA}}{T_1 + T_8} (d_{T_1} T_1 + d_{T_8} T_8)$$

$$\hspace{1cm} = \frac{P}{T_1 + T_8} \left[ (\lambda_{T_1 T_{10}} + \lambda_{T_1 T_{30}}) \frac{I_{12}}{K_{I_{12}} + I_{12}} + (\lambda_{T_8 T_{10}} + \lambda_{T_8 T_{30}}) \frac{I_{2}}{K_{I_{2}} + I_{2}} \right] \times \frac{1}{1 + Q/K_{TQ}} - \rho_p (d_{T_1} T_1 + d_{T_8} T_8) - \frac{\mu_{PA}}{T_1 + T_8} (d_{T_1} T_1 + d_{T_8} T_8) - \frac{\mu_{PA}}{T_1 + T_8} (d_{T_1} T_1 + d_{T_8} T_8)$$

where $\mu_{PA}$ represents the rate at which $P$ is depleted/blacked by $A$.

PD-L1 is expressed on the surface of activated CD4$^+$ and CD8$^+$ T cells [14, 15] and on cancer cells [15, 16]. Hence, the concentration of PD-L1 ($L$) is proportional to ($T_1 + T_8$) and $C$:

$$L = \rho_L (T_1 + T_8 + \epsilon C),$$  \hspace{1cm} (10)

where $\epsilon$ depends on the specific type of tumor.
PD-L1 from T cells or cancer cells ligands to PD-1 on the plasma membrane of T cells, thus forming a complex PD-1-PD-L1 (Q) on the T cells [15, 16]. Denoting the association and dissociation rates of Q by $\alpha_{PL}$ and $d_Q$, respectively, we can write

$$P + L \overset{\alpha_{PL}}{\underset{d_Q}{\rightleftarrows}} Q,$$

so that

$$\frac{\partial Q}{\partial t} + \nabla \cdot (uQ) - \delta_Q \nabla^2 Q = \alpha_{PL}PL - d_QQ.$$

The half-life of Q is less then 1 second (i.e. $1.16 \times 10^{-5}$ day) [33], and hence $d_Q$ is very large, and we may approximate the dynamical equation by the steady state equation, $\alpha_{PL}PL = d_QQ$, or

$$Q = \sigma PL,$$

where $\sigma = \alpha_{PL}/d_Q$.

**Equation for anti-PD-1 (A).** We assume that anti-PD-1 is injected intradermally every 3 days for 30 days (as in mouse experiments [21]) providing a source $\hat{A}(t)$ of anti-PD-1:

$$\hat{A}(t) = \begin{cases} 
\gamma_A & \text{if } t \leq 30, \\
\gamma_A \times \frac{33-t}{3} & \text{if } 30 < t \leq 33, \\
0 & \text{if } t > 33.
\end{cases}$$

where $\gamma_A$ is the effective level of the drug; although the level of the drug varies between injections, for simplicity we take it to be constant. The drug $A$ is depleted in the process of blocking PD-1. Hence,

$$\frac{\partial A}{\partial t} - \delta_A \nabla^2 A = \hat{A}(t) - \mu_{PA} \overset{\text{depletion through blocking PD-1}}{\underset{\text{degradation}}{A}},$$

where $\mu_{PA}$ is the rate at which $A$ is degraded in the process of blocking PD-1.

**Equation for cell velocity ($u$):** We assume that most of the tumor consists of the extracellular matrix, ECM (approximately, 0.6 $g/cm^3$), and cancer cells (approximately, $C = 0.4 g/cm^3$), and that the densities of $D$, $T_j$ and $T_k$ are approximately $4 \times 10^{-4}$, $2 \times 10^{-3}$ and $1 \times 10^{-3} g/cm^3$, respectively (as explained in the section on parameter estimation). We further assume that all cells are approximately of the same volume and surface area, so that their diffusion coefficients are the same. For definiteness, we take the constant in Eq (1) to be 0.4034. Adding Eqs (2)–(5), we then get

$$0.4034 \times \nabla \cdot u = \sum_{j=2}^{5} \text{RHS of Eq. (j)}.$$

To simplify the computations, we shall assume that the tumor is spherical and denote its moving boundary (i.e. its radius) by $r = R(t)$. We also assume that all the densities and concentrations are radially symmetric, that is, functions of $(r, t)$, where $0 \leq r \leq R(t)$. In particular, $u = u(r, t)e_r$, where $e_r$ is the unit radial vector, and each of the equations of the form

$$\frac{\partial X}{\partial t} + \nabla \cdot (uX) - \delta_X \nabla^2 X = F,$$
with $X = X(r, t)$, takes the form
\[
\frac{\partial X}{\partial t} + \frac{1}{r^2} \frac{\partial}{\partial r} (r^2 u X) - \delta X \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial X}{\partial r} \right) = F.
\]

**Equation for free boundary ($R$):** We assume that the free boundary $r = R(t)$ moves with the velocity of cells, so that
\[
\frac{dR(t)}{dt} = u(R(t), t).
\] (15)

**Boundary conditions** We assume that the naive CD4$^+$ T cells and naive CD8$^+$ T cells which migrated from the lymph nodes into the tumor microenvironment have constant densities $\hat{T}_1$ and $\hat{T}_8$ at the tumor boundary, and that they are activated by IL-12 upon entering the tumor. We represent this process by the flux conditions at the boundary $r = R(t)$:
\[
\frac{\partial T_1}{\partial r} + \sigma_T(I_{12})(T_1 - \hat{T}_1) = 0, \quad \frac{\partial T_8}{\partial r} + \sigma_T(I_{12})(T_8 - \hat{T}_8) = 0,
\] (16)
where $\sigma_T(I_{12}) = \frac{I_{12}}{I_{12} + K_{I_{12}}}$.

We impose the no-flux boundary conditions for all the remaining variables:
\[
\text{No-flux for } D(r, t), C(r, t), G(r, t), L_2(r, t), I_2(r, t), P(r, t) \text{ and } A(r, t), \quad \text{at } r = R(t).
\] (17)

It is tacitly assumed here that the receptors PD-1 and ligands PD-L1 become active only after the T cells are inside the tumor.

**Initial conditions.** We take initial values of all the variables to be constant. Later on we shall compare the simulations of the model with mouse experimental results, for 60 days. Accordingly, we take initial values whereby the average density of cancer cells has not yet increased to its steady state, 0.4 g/cm$^3$, and, in view of Eq (1), the total density of the immune cells is initially above its steady state. We take (in unit of g/cm$^3$):
\[
D = 6 \times 10^{-4}, \quad T_1 = 4 \times 10^{-4}, \quad T_8 = 2 \times 10^{-3}, \quad C = 0.3968.
\] (18)
We assume that initially $A = 0$, and
\[
G = 2.61 \times 10^{-10} \text{ g/cm}^3, \quad I_{12} = 1.8 \times 10^{-10} \text{ g/cm}^3, \quad I_2 = 4.74 \times 10^{-11} \text{ g/cm}^3, \quad P = 1.12 \times 10^{-10} \text{ g/cm}^3.
\]
These values are close to the steady state values which are computed in the section on parameter estimation.

**Results**

The simulations of the model were performed by Matlab based on the moving mesh method for solving partial differential equations with free boundary [34] (see the section on computational method). All the computations are done in dimensionless form, but displayed in dimensional form.

The average density or concentration of a species is defined as its total mass in the tumor divided by the tumor volume. Fig 2 shows the average concentrations of all the species of the model over a period of 60 days in the control case, that is, when no drugs are administered; the parameter values are given in Tables 2 and 3. The radius of the tumor is increasing, from 0.01
cm to 0.0313 cm. The average density of the cancer cells is initially increasing and later it stabilizes while the densities of the immune cells are first decreasing and later stabilize. Correspondingly, the concentrations of the cytokines produced by the immune system also first decrease and later stabilize. Some of the parameters in the model were estimated by assuming the immune cells and cytokines are in steady state. Their steady states in Fig 2 approximately agree with those which we assumed in estimating the parameters, thus establishing the consistency of our assumed steady-state values.

We can also simulate the spatial distribution of each of the variables. Fig 3 shows the distribution of cancer cells and T cells ($T_1 + T_8$) at times $t = 15, 30, 60$ days. We see that the density of T cells increases toward the boundary; this is a result of the influx of T cells from the lymph nodes. Correspondingly, the density of cancer cells decreases toward the boundary. In our model, we tacitly assumed avascular conditions, since we wanted to focus primarily on the difference between control and treatment. Since, however, the tumor radius reaches approximately 313 $\mu m$ at day 60, hypoxic conditions may actually reduce the cancer cells’ density at the core of the tumor (both in control and treatment).

In Figs 2 and 3, we have taken $\varepsilon = 0.01$. Some cancer cells may express more or less PD-L1 [17, 35–37]. By decreasing or increasing $\varepsilon$, the radius of the tumor will decrease or increase, respectively, but the profiles of the densities/concentrations remain qualitatively the same (not shown here).

We next proceed to explore the effect of treatment with GM-CSF-secreting vaccine (GVAX) and anti-PD-1 drug. We are unable to make a direct connection between the levels of
Table 2. Summary of parameter values.

| Notation | Description | Value used | References |
|----------|-------------|------------|------------|
| $\delta_D$ | diffusion coefficient of DCs | $8.64 \times 10^{-7}$ cm$^2$ day$^{-1}$ | [59] |
| $\delta_{T_i}$ | diffusion coefficient of CD4$^+$ T cells | $8.64 \times 10^{-7}$ cm$^2$ day$^{-1}$ | [59] |
| $\delta_{T_e}$ | diffusion coefficient of CD8$^+$ T cells | $8.64 \times 10^{-7}$ cm$^2$ day$^{-1}$ | [59] |
| $\delta_C$ | diffusion coefficient of tumor cells | $8.64 \times 10^{-7}$ cm$^2$ day$^{-1}$ | [59] |
| $\delta_{GM}$ | diffusion coefficient of GM-CSF | $9.8495 \times 10^{-10}$ cm$^2$ day$^{-1}$ | estimated |
| $\delta_{IL-12}$ | diffusion coefficient of IL-12 | $6.0472 \times 10^{-8}$ cm$^2$ day$^{-1}$ | estimated |
| $\delta_{IL-2}$ | diffusion coefficient of IL-2 | $9.9956 \times 10^{-8}$ cm$^2$ day$^{-1}$ | estimated |
| $\delta_A$ | diffusion coefficient of anti-PD-1 | $7.85 \times 10^{-8}$ cm$^2$ day$^{-1}$ | estimated |
| $\sigma_0$ | flux rate of T cells on the boundary | $1$ cm$^{-1}$ | [59] |
| $\lambda_{DC}$ | activation rate of DCs by GM-CSF | $20.02$ day$^{-1}$ | [65, 66] & estimated |
| $\lambda_{DC_t}$ | activation rate of DCs by tumor cells | $0.364$ day$^{-1}$ | estimated |
| $\lambda_{T_i,f_2}$ & activation rate of CD4$^+$ T cells by IL-12 | $4.66$ day$^{-1}$ | estimated |
| $\lambda_{T_i,f_2}$ & activation rate of CD4$^+$ T cells by IL-2 | $0.25$ day$^{-1}$ | estimated |
| $\lambda_{T_e,f_2}$ & activation rate of CD8$^+$ T cells by IL-12 | $4.15$ day$^{-1}$ | estimated |
| $\lambda_{T_e,f_2}$ & activation rate of CD8$^+$ T cells by IL-2 | $0.25$ day$^{-1}$ | [59] |
| $\lambda_C$ | growth rate of cancer cells | $0.616$ day$^{-1}$ | [59] |
| $\lambda_{GM}$ | non-vaccine source of GM-CSF | $2.23 \times 10^{-10}$ g/cm$^3$ · day | estimated |
| $\lambda_{IL-12}$ | production rate of IL-12 by DCs | $5.18 \times 10^{-7}$ day$^{-1}$ | estimated |
| $\lambda_{IL-2}$ | production rate of IL-2 by CD4$^+$ T cells | $2.82 \times 10^{-7}$ day$^{-1}$ | estimated |
| $n_1$ | killing rate of tumor cells by CD4$^+$ T cells | $11.5$ day$^{-1}$ · cm$^{-3}$/g | estimated |
| $n_2$ | killing rate of tumor cells by CD8$^+$ T cells | $46$ day$^{-1}$ · cm$^{-3}$/g | estimated |
| $\mu_{PA}$ & blocking rate of PD-1 by anti-PD-1 | $6.87 \times 10^{6}$ cm$^3$/g · day | estimated |
| $\rho_P$ & expression of PD-1 in T cells | $2.49 \times 10^{-7}$ | estimated |
| $\rho_L$ & expression of PD-L1 in T cells | $5.22 \times 10^{-7}$ | estimated |
| $\xi$ & expression of PD-L1 in tumor cells | $0 - 0.01^*$ | estimated |
| $d_D$ & death rate of DCs | $0.1$ day$^{-1}$ | [59] |
| $d_{T_i}$ & death rate of CD4$^+$ T cells | $0.197$ day$^{-1}$ | [59] |
| $d_{T_e}$ & death rate of CD8$^+$ T cells | $0.18$ day$^{-1}$ | [59] |
| $d_C$ & death rate of tumor cells | $0.17$ day$^{-1}$ | [59] |
| $d_{GM}$ & degradation rate of GM-CSF | $1.28$ day$^{-1}$ | [65] |
| $d_{IL-12}$ & degradation rate of IL-12 | $1.38$ day$^{-1}$ | [59] |
| $d_{IL-2}$ & degradation rate of IL-2 | $2.376$ day$^{-1}$ | [59] |
| $d_A$ & degradation rate of anti-PD-1 | $0.0462$ day$^{-1}$ | [42] |

* In the simulations we took $\varepsilon = 0.01$.

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Drugs administered to the patient, and their ‘effective strengths’ $\gamma_C$ and $\gamma_A$ in the model, since these data are not available. Based on the estimate of the concentration of GM-CSF in normal healthy tissue (see Parameter Estimations for Eq (6)), we chose the order of magnitude of GVAX to be $10^{-10}$ (g/cm$^3$ · day). The order of magnitude for anti-PD-1 drug ($\gamma_A$) is chosen so as to get the best agreement with the mouse experiments. In Fig 4(a), the ‘effective strength’ of the vaccine is given by $\gamma_C = 0.87 \times 10^{-10}$ g/cm$^3$ · day and the ‘effective strength’ of the anti-PD-1 is given by $\gamma_A = 2 \times 10^{-10}$ g/cm$^3$ · day. We see that, as a single-agent, anti-PD-1 is more effective than GVAX, and with the combined therapy the tumor radius is still increasing. This is in agreement with the mouse experiments reported in Fig 1-(A) (with melanoma) in [21], and Figs 3-(b) (with colon carcinoma) and 3-(c) (with melanoma) in [22].
**Table 3. Summary of parameter values.**

| Parameter          | Value                                      | Source          |
|--------------------|--------------------------------------------|-----------------|
| $K_G$              | half-saturation of GM-CSF                  | $1.74 \times 10^{-9}$ g/cm$^3$ | [65] |
| $K_C$              | half-saturation of tumor cells             | $0.4$ g/cm$^3$  | [59] |
| $K_{IL-12}$        | half-saturation of IL-12                   | $1.5 \times 10^{-10}$ g/cm$^3$ | [59] |
| $K_{IL-2}$         | half-saturation of IL-2                    | $2.37 \times 10^{-11}$ g/cm$^3$ | [59] |
| $K_{CD4+}$         | half-saturation of CD4$^+$ T cells         | $2 \times 10^{-3}$ g/cm$^3$ | estimated |
| $K_{CD8+}$         | half-saturation of CD8$^+$ T cells         | $1 \times 10^{-3}$ g/cm$^3$ | estimated |
| $K_{PD-L1}$        | inhibition of function of T cells by PD-1-PD-L1 | $1.365 \times 10^{-18}$ g/cm$^3$ | estimated |
| $D_0$              | density of immature DCs                   | $2 \times 10^{-5}$ g/cm$^3$ | [59] |
| $T_{CD4+}$         | density of naive CD4$^+$ T cells           | $4 \times 10^{-3}$ g/cm$^3$ | estimated |
| $T_{CD8+}$         | density of naive CD8$^+$ T cells           | $2 \times 10^{-3}$ g/cm$^3$ | estimated |
| $C_M$              | carrying capacity of cancer cells          | $0.8$ g/cm$^3$  | [59] |
| $T_{CD4+}$         | density of CD4$^+$ T cells from lymph node | $4 \times 10^{-3}$ g/cm$^3$ | estimated |
| $T_{CD8+}$         | density of CD8$^+$ T cells from lymph node | $2 \times 10^{-3}$ g/cm$^3$ | estimated |
| $Y_G$              | source of GM-CSF from the vaccine          | $1 \times 10^{-10}$ g/cm$^3$ · day$^*$ | estimated |
| $Y_A$              | source of anti-PD-1                        | $1 \times 10^{-10}$ g/cm$^3$ · day$^*$ | estimated |

* Values used in sensitivity analysis.

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**Fig 3. Spatial distribution of density of cancer cells and $T_1 + T_6$ cells at time $t = 15, 30, 60$ days.** All parameter values are the same as in Fig 2.

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In Fig 4(b), we increased $\gamma_A$ by a factor 3/2. As a result, the tumor radius begins to decrease around day 50, even when administering anti-PD-1 as single-agent. This is in agreement with mouse experiments (with colon carcinoma) reported in Fig 1-(D) of [23].

In Fig 4(a), GM-CSF-secreting vaccine alone did not reduce the tumor radius as much as anti-PD-1 alone. However, if we increase the strength of the vaccine and decrease the anti-PD-1, taking for example, $\gamma_G = 3.84 \times 10^{-10}$ g/cm$^3$·day and $\gamma_A = 1 \times 10^{-10}$ g/cm$^3$·day, we then find that the vaccine decreases the tumor radius more than anti-PD-1 does; this is shown in Fig 5, and it is in agreement with mouse experiments (with melanoma) reported in Fig 1-(B) of [23].
The results of Figs 4 and 5 show that a combination therapy of GVAX and anti-PD-1 in appropriate amounts could significantly slow the growth of a tumor.

There is some uncertainty in the estimates of some of the parameters of the model. With somewhat different choices of these parameters, the simulation results will change quantitatively, and sensitivity analysis indicates the direction and intensity of the change (see the section on sensitivity analysis). In particular, the choice of $\gamma_G$ and $\gamma_A$ will affect the relative reduction in the growth of the tumor radius. In Figs 4 and 5 we made specific choices of $\gamma_G$ and $\gamma_A$, in order to get simulations that agree with experimental results.

We next consider combination therapy for any values of GVAX and anti-PD-1. We define the efficacy of the combination therapy with $(\gamma_G, \gamma_A)$ by the formula:

$$E(\gamma_G, \gamma_A) = \frac{R_{60}(0, 0) - R_{60}(\gamma_G, \gamma_A)}{R_{60}(0, 0)},$$

where $R_{60} = R_{60}(\gamma_G, \gamma_A)$ represents the tumor radius computed at day 60. If the tumor radius at day 60, $R_{60}(\gamma_G, \gamma_A)$, is smaller than the radius in the control case, $R_{60}(0, 0)$, then the efficacy is a positive number, and its value is between 0 and 1 (or between 0% and 100%); the efficacy increases to 1 (or to 100%) when the tumor radius $R_{60}(\gamma_G, \gamma_A)$ decreases to 0 by day 60. Fig 6 (a) is the efficacy map of the combined therapy with $\gamma_G$ in the range of $0 - 4.8 \times 10^{-10}$ g/cm$^3$ · day and $\gamma_A$ in the range of $0 - 2 \times 10^{-10}$ g/cm$^3$ · day. For a given $\gamma_G$, the optimal synergy of the combined therapy $(\gamma_G, \gamma_A)$ occurs when $(\gamma_G, \gamma_A)$ lies on the solid curve.

The results of Figs 4 and 5 show that a combination therapy of GVAX and anti-PD-1 in appropriate amounts could significantly slow the growth of a tumor.

Fig 6. Drug efficacy map and synergy map. (a) Efficacy map: The color column shows the efficacy $E(\gamma_G, \gamma_A)$ when $\gamma_G$ varies between $0 - 4.8 \times 10^{-10}$ g/cm$^3$ · day and $\gamma_A$ varies between $0 - 4 \times 10^{-10}$ g/cm$^3$ · day. (b) Synergy map: The color column shows the synergy $\sigma(\gamma_G, \gamma_A)$ when $\gamma_G$ varies between $0 - 2.4 \times 10^{-10}$ g/cm$^3$ · day and $\gamma_A$ varies between $0 - 2 \times 10^{-10}$ g/cm$^3$ · day. For a given $\gamma_G$, the optimal synergy of the combined therapy $(\gamma_G, \gamma_A)$ occurs when $(\gamma_G, \gamma_A)$ lies on the solid curve.

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Early stage clinical trials consider the safety of each of the drugs, GVAX and anti-PD-1, separately. In the GVAX treatment of patients with pancreatic cancer, no dose-limiting toxicity or minimal toxicity was observed [38–40]. On the other hand, in treatment with anti-PD-1, mild to moderate toxicity (grades 1 and 2) was observed in melanoma [41, 42]. In non-small-cell lung cancer, 14% of the patients were observed to have more severe toxicity (grades 3 and 4) [43]. Based on these observations we conclude that anti-PD-1 causes more toxicity than...
GVAX. However, clinical trials on the safety and efficacy of the combined drugs are limited [23, 44, 45].

The amount of drug in clinical trials is constrained by the maximum tolerated dose (MTD). In combination therapy this constraint may depend on the proportion between the amounts of the drugs. We note that there is a large literature on the trade-off between efficacy and toxicity [46–49]. Here we consider, as an example, two treatments, of the drugs. We note that there is a large literature on the trade-off between efficacy and toxicity [23, 44, 45]. However, clinical trials on the safety and efficacy of the combined drugs are limited.

In combination therapy this constraint may depend on the proportion between the amounts of the drugs. We note that there is a large literature on the trade-off between efficacy and toxicity [46–49]. Here we consider, as an example, two treatments, of the drugs. We note that there is a large literature on the trade-off between efficacy and toxicity [23, 44, 45]. However, clinical trials on the safety and efficacy of the combined drugs are limited. We compare treatment of combination (γ₆, γ₄) with monotherapy GVAX and monotherapy anti-PD-1. For monotherapy with GVAX, we take (1 + θ₆)γ₆, and for monotherapy with anti-PD-1 we take (1 + θ₄)γ₄, with θ₆ < θ₄, to reflect the higher toxicity associated with anti-PD-1. If E(γ₆, γ₄) is larger than both E((1 + θ₆)γ₆, 0) and E(0, (1 + θ₄)γ₄), then we say that the synergy for the combination (γ₆, γ₄) is positive, and otherwise, we say that the synergy is negative. More generally, we define the synergy σ = σ(γ₆, γ₄) by the formula:

$$\sigma(\gamma_6, \gamma_4) = \frac{E(\gamma_6, \gamma_4)}{\max\{E((1 + \theta_6)\gamma_6, 0), E(0, (1 + \theta_4)\gamma_4)\}} - 1.$$  \hspace{1cm} (19)

Thus σ(γ₆, γ₄) > 0 (positive synergy) if the combination (γ₆, γ₄) reduces tumor growth more than either one of the single agents with (1 + θ₆)γ₆ or (1 + θ₄)γ₄. Negative synergy occurs in the reverse case where instead of a combination therapy with (γ₆, γ₄) we achieve better reduction of the tumor radius Rₜ if we apply only one drug, (1 + θ₆)γ₆ or (1 + θ₄)γ₄. The above concept of synergy is somewhat different from the usual definitions of synergy. For definiteness we take θ₆ = 1 and θ₄ = 0.5, but this choice, which is somewhat arbitrary, could be made more precise as more clinical data become available.

Fig 6(b) is the synergy map for (γ₆, γ₄) in the same range as in Fig 6(a); the color column shows the synergy σ(γ₆, γ₄), with values that vary from -0.38 to 0.28.

We first note that the synergy is negative if γ₆ < 0.2 × 10⁻¹⁰ g/cm³ day. The reason is the following: if γ₆ is small then the numbers of T cells is also small, so instead of introducing a drug γ₄ which blocks the relatively small number of PD-1, it is more effective to increase the number of T cells by increasing γ₆, i.e. E(2γ₆, 0) > E(γ₆, γ₄).

Next, for any fixed γ₆, as seen in Fig 6(b), the synergy first increases with γ₄ and then decreases. In order to explain this occurrence, we note that with γ₆ fixed, the number of T cells that arrive into the tumor microenvironment is limited, and so is the number of their PD-1. Thus, in order to block the PD-1 there is a need for only a limited amount of anti-PD-1 drug; i.e. it is ‘wasteful’ to administer too much of γ₄. We conclude that the maximum synergy is achieved when the amount of γ₄ is appropriately dependent on the amount of γ₆, as indicated by the solid curve shown in Fig 6(b).

Fig 7 shows that as the amounts of γ₆ and γ₄ increase the average density of T cells (T₁ + Tₚ) increases, and correspondingly the average density of cancer cells decreases. We also see that the density of T cells shown in the color column increases approximately by a factor of 6, whereas the density of cancer cells decreases approximately by a factor of 1.075. The proportion of these changes (i.e. 5/0.075) is similar to the proportion of densities of cancer cells to T cells.

**Conclusion**

The introduction of immune checkpoint inhibitors has been a very promising approach to cancer treatment. Blockage of the programmed death PD-1/PD-L1 is increasingly explored in
single-agent studies [16, 18]. However, because of the lack of tumor-infiltrating effector T cells, many patients do not respond to checkpoint inhibitor treatment as single-agent [18]. On the other hand, cancer vaccines have been shown to induce effector T-cells infiltration into tumors [19], although, to be fully effective, cancer vaccines have to overcome immune evasion [10]. It was recently suggested that the combination of a cancer vaccine and an immune checkpoint inhibitor may function synergistically to induce more effective antitumor immune response [18, 20]. Clinical trials to test such combinations are currently ongoing [18, 20].

In the present paper we developed a mathematical model to study the efficacy of the combination of a GM-CSF-secreting cancer vaccine (GVAX) and an anti-PD-1 drug, and to address the following question: at what proportion should the two drugs be administered in order to achieve the best efficacy when the amount of drugs is limited by MTD. The mathematical model is represented by a system of partial differential equations, based on the interactions among cancer cells, dendritic cells, $CD4^+$ and $CD8^+$ T cells, cytokines IL-12 and IL-2, GM-CSF, PD-1, PD-L1, PD-1-PD-L1 complex and anti-PD-1. Simulations of the model are shown to be in qualitative agreement with mouse experiments [21–23].

The cancer vaccine and the anti-PD-1 work in collaboration: The vaccine increases the number of tumor-infiltrating effector T cells and the anti-PD-1 ensures that these cells remain active. Given a fixed amount $\gamma_G$ of the vaccine and $\gamma_A$ of the anti-PD-1, it is important to estimate the level of synergy between these amounts in order to administer them in the most effective proportion. We introduced a specific concept of synergy $\sigma(\gamma_G, \gamma_A)$ and developed accordingly a synergy map in Fig 6(b). The map shows that if $\gamma_G$ is small, single-agent treatment (with $\gamma_A = 0$) is the best treatment. Fig 6(b) also shows that, for any $\gamma_G$, there is a unique value $\gamma_{AG}$ such that the synergy increases as $\gamma_A$ increases as long as $\gamma_A$ remains smaller than $\gamma_{AG}$, but the synergy decreases as $\gamma_A$ increases above $\gamma_{AG}$; the points $(\gamma_G, \gamma_{AG})$ form the solid curve shown in Fig 6(b). We suggest that for optimal efficacy under MTD constraint, the level of dosage of anti-PD-1 ($\gamma_A$) should be related to the level of dosage of GVAX ($\gamma_G$) by setting $\gamma_A = \gamma_{AG}$ as indicated by the solid curve in Fig 6(b).

The mathematical model presented in this paper has several limitations:
1. In order to focus on the combined therapy of a cancer vaccine and an anti-PD-1 drug, we did not include some other important cells and cytokines that are found in the tumor microenvironment, such as T regulatory cells, macrophages, endothelial cells, and IL-10, IL-6 and TGF-β. We also did not include blood vessels and oxygen, thus assuming that the tumor is avascular. We tacitly assumed that the effect of these omissions is not significant in comparing the results of therapy to no therapy.

2. We assumed that the densities of immature, or naive, immune cells remain constant throughout the progression of the cancer and that dead cells are quickly removed from the tumor.

3. In estimating parameters we made a steady state assumption in some of the differential equations.

4. In the definition of synergy, and in the synergy map, we included in a crude way the fact that anti-PD-1 causes more toxicity than GVAX. Our aim was to develop a concept that will take account not only of efficacy but also of toxicity. For this reason we compared the treatment benefits for combination ($\gamma_G$, $\gamma_A$) with the single agents ($2\gamma_G$, 0) and (0, $1.5\gamma_A$).

5. We did not make any direct connection between drugs administered to the patient, and their ‘effective strengths’ $\gamma_G$ and $\gamma_A$, as they appear in the differential equations, since these data are not available. The order of magnitude of GVAX ($10^{-10}$) was based on the estimate of the concentration of GM-CSF in normal healthy tissue (see Parameter Estimations for Eq (6)). We simulated the model with different orders of magnitude for anti-PD-1 drug ($\gamma_A$) and found the best agreement with the mouse experiments (in Figs 4 and 5) when $\gamma_A$ is also of order of magnitude $10^{-10}$.

6. Although our mathematical model does not presume any geometric form of the tumor, for simplicity, the simulations have been carried out only in the case of a spherical tumor. We note however that spherical cancer models have been used in research as an intermediate between in vitro cancer line cultures and in vivo cancer [50]. Furthermore, spheroids mirror the 3D cellular context and therapeutically relevant pathophysiological gradient of in vivo tumors [51].

Clinical data on efficacy and toxicity are quite limited at this time. Our model should be viewed as setting up a computational framework, to address the question of optimal efficacy in combination therapy with cancer vaccine and checkpoint inhibitor.

Materials and methods

Parameter estimation

Half-saturation. In an expression of the form $Y = \frac{X}{K_X + X}$ where $Y$ is activated by $X$, the half-saturation parameter $K_X$ is taken to be the approximate steady state concentration of species $X$.

Diffusion coefficients. By [52], we have the following relation for estimating the diffusion coefficients of a protein $p$:

$$\delta_p = \frac{M_p^{1/3}}{M_\nu^{1/3}} \delta_\nu,$$

where $M_p$ and $\delta_\nu$ are respectively the molecular weight and diffusion coefficient of VEGF, $M_p$ is the molecular weight of $p$, and $M_\nu = 24\text{kDa}$ [53] and $\delta_\nu = 8.64 \times 10^{-2} \text{ cm}^2 \text{ day}^{-1}$ [54]. Since $M_G = 15.5\text{kDa}$, $M_{I_2} = 70\text{kDa}$, $M_I = 16.2\text{kDa}$ [55] and $M_A = 32\text{kDa}$ [56], we get $\delta_G = \ldots$
9.9956 × 10^{-2} \text{cm}^2 \text{day}^{-1}, \delta_{D_1} = 6.0472 \times 10^{-2} \text{cm}^2 \text{day}^{-1}, \delta_G = 9.8495 \times 10^{-2} \text{cm}^2 \text{day}^{-1}, \text{and} \delta_A = 7.85 \times 10^{-2} \text{cm}^2 \text{day}^{-1}.

Eq (2): The number of DCs in various organs (heart, kidney, pancreas and liver) in mouse varies from 1.1 × 10^6 cells/cm^3 to 6.6 × 10^6 cells/cm^3 [57]. Mature DCs are approximately 10 to 15 μm in diameter [58]. Accordingly, we estimate steady states of DCs by $K_D = 4 \times 10^{-4} \text{g/cm}^3$. We assume that the density of immature DCs is smaller than the actived DCs, and take $D_0 = \frac{1}{20} K_D = 2 \times 10^{-5} \text{g/cm}^3$. We also assume that the activation of DCs by GM-CSF-secreting vaccine is more effective than their activation by NCs-secreted HMGB-1, and take $\lambda_{DG} = 10 \times \frac{C_D}{C_G + C_D}$. From the steady state of Eq (2) in the control case (with no drug), we get

$$\lambda_{DC} D_0 \cdot \frac{C}{K_C + C} + \lambda_{DG} D_0 \cdot \frac{G}{K_G + G} - d_D D = 0,$$

where $d_D = 0.1/\text{day}$ [59], $D_0 = 2 \times 10^{-5} \text{g/cm}^3$, $D = K_D = 4 \times 10^{-4} \text{g/cm}^3$, $C = K_C = 0.4 \text{g/cm}^3$, and $G = K_G = 1.74 \times 10^{-10} \text{g/cm}^3$ and $K_G = 1.74 \times 10^{-9} \text{g/cm}^3$ ($K_G$ and $K_G$ are estimated together with other estimates of Eq (6)). Hence $\lambda_{DC} = 0.364/\text{day}$ and $\lambda_{DG} = 20.02/\text{day}$.

Eq (3): The number of lymphocytes is approximately twice the number of DCs [57]. T cells are approximately 14 to 20 μm in diameter. Assuming that the number of Th1 cells is 1/4 of the number of lymphocytes, we estimate the steady state density of Th1 cells by $K_T = 2 \times 10^{-3} \text{g/cm}^3$. We assume the density of naive CD4⁺ T cells to be less than the density of Th1, and take $T_{i0} = \frac{1}{2} K_T = 4 \times 10^{-4} \text{g/cm}^3$. We also assume that in steady state, $Q/K_{TQ} = 2 (K_{TQ} is estimated together with other estimates of Eqs (9)–(12)). From the steady state of Eq (3), we get

$$\left(\lambda_{T_{i1}T_{i2}} T_{i0} \cdot \frac{1}{2} + \lambda_{T_{i1}T_i} T_i \cdot \frac{1}{2} \right) \cdot \frac{1}{3} - d_{T_i} T_i = 0,$$

where $T_{i0} = 4 \times 10^{-4} \text{g/cm}^3$, $T_i = K_{T_i} = 2 \times 10^{-3} \text{g/cm}^3$, and, by [59], $\lambda_{T_{i1}T_{i2}} = 0.25/\text{day}$ and $d_{T_i} = 0.197/\text{day}$. Hence $\lambda_{T_{i1}T_{i2}} = 4.66/\text{day}$.

Eq (4): The CD4/CD8 ratio in the blood is 2:1. We assume a similar ratio in the tissue, and take $T_{s0} = \frac{1}{2} T_{i0} = 2 \times 10^{-4} \text{g/cm}^3$. We also take the steady state of $T_s$ to be half of the steady state of $T_i$, i.e., $K_{T_s} = \frac{1}{2} K_{T_i} = 1 \times 10^{-3} \text{g/cm}^3$. From the steady state equation of Eq (4), we get

$$\left(\lambda_{T_{s1}T_{s2}} T_{s0} \cdot \frac{1}{2} + \lambda_{T_{s1}T_s} T_s \cdot \frac{1}{2} \right) \cdot \frac{1}{3} - d_{T_s} T_s = 0,$$

where $T_{s0} = 5 \times 10^{-5} \text{g/cm}^3$, $T_s = K_{T_s} = 1 \times 10^{-3} \text{g/cm}^3$, and, by [59], $\lambda_{T_{s1}T_{s2}} = 0.25/\text{day}$ and $d_{T_s} = 0.18/\text{day}$. Hence $\lambda_{T_{s1}T_{s2}} = 4.15/\text{day}$.

Eq (5): We take $d_C = 0.17/\text{day}$ and $C_M = 0.8 \text{g/cm}^3$ [59]. In the absence of immune responses and anti-tumor drugs, the tumor grows according to

$$\frac{dC}{dt} = \lambda_C \left(1 - \frac{C}{C_M}\right) - d_C C,$$

and with immune response, the tumor grows according to

$$\frac{dC}{dt} = \lambda_C \left(1 - \frac{C}{C_M}\right) - (\eta_1 T_1 + \eta_s T_s) C - d_C C.$$
Mouse experiments show that the tumor volume on average doubles within 5-15 days [21–23]. Assuming a linear growth

\[
\frac{dC}{dt} = \lambda_0 C, \quad \text{where } \lambda_0 > 0,
\]
during the volume doubling time, we conclude from Eq (21) that

\[
\lambda_c C \left(1 - \frac{C}{C_M}\right) - (\eta_1 T_1 + \eta_8 T_8) C - d_c C = \lambda_0 C.
\]  

(22)

where \(\lambda_0 \in \left(\frac{\mu m}{day}, \frac{mm}{day}\right)\). We assume that with no immune responses

\[
\frac{dC}{dt} = 2\lambda_0 C,
\]
so that, by Eq (20), we get

\[
\lambda_c C \left(1 - \frac{C}{C_M}\right) - d_c C = 2\lambda_0 C.
\]  

(23)

We take \(\lambda_0 = 0.069/\text{day}\), and assume that in steady state of Eqs (20) and (21), \(C\) is approximately 0.4 g/cm\(^3\), so that from Eq (23) we get

\[
\frac{1}{2} \lambda_c - d_c = 2\lambda_0,
\]
or \(\lambda_c = 0.616/\text{day}\). From Eqs (23) and (22), we get \(\eta_1 T_1 + \eta_8 T_8 = \lambda_0\). Noting that \(T_8\) cells kill cancer cells more effectively than \(T_1\) cells, we take \(\eta_8 = 4\eta_1\), so that \(\eta_1 = \frac{\eta_8}{\eta_8 + \eta_8} = 11.5 \text{ cm}^3/\text{g} \cdot \text{day}\) and \(\eta_8 = 46 \text{ cm}^3/\text{g} \cdot \text{day}\) (with \(T_1 = K_{T_1} = 2 \times 10^{-3} \text{ g/cm}^3\) and \(T_8 = K_{T_8} = 1 \times 10^{-3} \text{ g/cm}^3\) as in the estimates for Eqs (3) and (4)).

Eq (6): With no drugs, the steady state plasma concentration of GM-CSF is \(G = \hat{K}_G = 1.74 \times 10^{-10} \text{ g/cm}^3\) [60]. The half-life of GM-CSF is 13 hours [61], i.e., 0.54 day, so that \(d_c = \frac{\ln 2}{0.54} = 1.28/\text{day}\). From the steady state of Eq (6) (with no drugs), we get \(\hat{\lambda}_G = d_c G = d_c \hat{K}_G = 2.23 \times 10^{-10} \text{ g/cm}^3 \cdot \text{day}\). But when the GM-CSF-secreting vaccine is administered, the concentration of GM-CSF shoots up, and we assume that at steady states it reaches the level of \(G = K_G = 10\hat{K}_G = 1.74 \times 10^{-9} \text{ g/cm}^3\).

Eq (7): From the steady state of Eq (7), we get \(\lambda_{I_1,D}D - d_{I_1}I_{12} = 0\), where \(d_{I_1} = 1.38/\text{day}\) [59] and \(I_{12} = K_{I_{12}} = 1.5 \times 10^{-10} \text{ g/cm}^3\) [59], and \(D = K_D = 4 \times 10^{-4} \text{ g/cm}^3\). Hence, \(\lambda_{I_1,D} = 5.18 \times 10^{-7}/\text{day}\).

Eq (8): From the steady state of Eq (8), we get \(\lambda_{I_1,T_1}T_1 - d_{I_2}I_2 = 0\), where \(d_{I_2} = 2.376/\text{day}\) [59] and \(I_2 = K_{I_2} = 2.37 \times 10^{-11} \text{ g/cm}^3\) [59], and \(T_1 = K_{T_1} = 2 \times 10^{-3} \text{ g/cm}^3\). Hence, \(\lambda_{I_1,T_1} = 2.82 \times 10^{-9}/\text{day}\).

Eqs (9)–(12): In order to estimate the parameter \(K_{TQ}\) (in Eqs (3) and (4)), we need to determine the steady state concentrations of \(P\) and \(L\) in the control case (no drugs). To do that, we need to estimate \(\rho_P\) and \(\rho_L\). By [62], the mass of one PD-1 protein is \(m_P = 8.3 \times 10^{-8} \text{ pg} = 8.3 \times 10^{-20} \text{ g}\), and by [63] the mass of one PD-L1 is \(m_L = 5.8 \times 10^{-8} \text{ pg} = 5.8 \times 10^{-20} \text{ g}\). We assume that the mass of one T cell is \(m_T = 10^{-7} \text{ g}\). By [14], there are 3000 PD-1 proteins and 9000 PD-L1 proteins on one T cell (\(T_1\) or \(T_2\)). Since \(\rho_P T\) is the density of PD-1 (without anti-PD-1 drug), we get \(\rho_P = 3000 \times \frac{m_P}{m_T} = \frac{3000 \times (8.3 \times 10^{-20})}{10^{-7}} = 2.49 \times 10^{-7}\) and \(\rho_L = 9000 \times \frac{m_L}{m_T} = \frac{9000 \times (5.8 \times 10^{-20})}{10^{-7}} = 5.22 \times 10^{-7}\).
In steady state, \( T_1 = 2 \times 10^{-3} \) g/cm\(^3\) and \( T_8 = 1 \times 10^{-3} \) g/cm\(^3\). Hence, in steady state,
\[
P = \rho_p(T_1 + T_8) = (2.49 \times 10^{-7}) \times (2 \times 10^{-3} + 1 \times 10^{-3}) = 7.47 \times 10^{-10} \text{ g/cm}^3.
\]

The parameter \( e \) in Eq (10) depends on the type of cancer; many cancer cells, but not all, express PD-L1 [17, 35–37]. Accordingly, we assume \( e \) varies in the interval 0–0.01, but in the simulations we take \( e = 0.01 \). Then, from Eq (10), we get
\[
L = \rho_L(T_1 + T_8 + eC) = (5.22 \times 10^{-7}) \times [2 \times 10^{-3} + 1 \times 10^{-3} + 0.01 \times 0.4] = 3.654 \times 10^{-9} \text{ g/cm}^3.
\]

In steady state with \( P = \tilde{P}, L = \tilde{L} \) and \( Q = \tilde{Q} \), we have, by Eq (12), \( \tilde{Q} = \sigma \tilde{P} \tilde{L} \). We take \( K_{TQ} = \frac{1}{2} \tilde{Q} = \frac{1}{2} \sigma \tilde{P} \tilde{L} \). Hence, \( Q/K_{TQ} = PL/(\frac{1}{2} \tilde{P} \tilde{L}) \) and
\[
\frac{1}{1 + Q/K_{TQ}} = \frac{1}{1 + PL/(\frac{1}{2} \tilde{P} \tilde{L})} = \frac{1}{1 + PL/K'_{TQ}},
\]
where \( K'_{TQ} := \frac{1}{2} \tilde{P} \tilde{L} = \frac{1}{2} \times (7.47 \times 10^{-10}) \times (3.654 \times 10^{-9}) = 1.365 \times 10^{-18} \text{ g}^2/\text{cm}^6 \).

Eq (13): By [42], the half-life of anti-PD-1 is 15 days, so that \( d_A = \frac{\ln 2}{15} = 4.62 \times 10^{-2} \text{ day}^{-1} \).
We assume that 10% of A is used in blocking PD-1, while the remaining 90% degrades naturally. Hence, in steady state, \( \mu_{PA} PA/10\% = d_A A/90\% \), so that
\[
\mu_{PA} = \frac{d_A}{9P} = \frac{4.62 \times 10^{-2}}{9 \times (7.47 \times 10^{-10})} = 6.87 \times 10^6 \text{ cm}^3/\text{g} \cdot \text{day}.
\]

If the percentage of A used in blocking PD-1 is changed, the value of \( \mu_{PA} \) will also change, but the results of simulations do not change qualitatively (not shown here).

**Sensitivity analysis**

We performed sensitivity analysis, with respect to the tumor radius \( R \) at day 60, with respect to some of the production parameters of the System (2)–(13), namely, \( \lambda_{DC}, \lambda_{DG}, \lambda_{T,1}, \lambda_{T,2}, \lambda_{T,3}, \lambda_{T,4}, \lambda_{I,1}, \lambda_{I,2}, \lambda_{I,3}, \lambda_{I,4}, \) and the important parameters \( K_{TQ}, \eta_1 \) and \( \eta_8 \). Following the method in [64], we performed Latin hypercube sampling and generated 1000 samples to calculate the partial rank correlation coefficients (PRCC) and the p-values with respect to the tumor radius at day 60. In sampling all the parameters, we took the range of each from 1/2 to twice its values in Tables 2 and 3. The results are shown in Fig 8.

Not surprisingly all the parameters are negatively correlated with the tumor radius. We note that the highest negatively correlated parameters are the activation rate of dendritic cells by cancer cells (\( \lambda_{DC} \)) and the inhibition of T cells activation by PD-1-PD-L1 complex (\( K_{TQ} \)). However, with different values of \( \gamma_G \) and \( \gamma_A \) the parameter \( \lambda_{DG} \) can exceed \( \lambda_{DC} \).

**Computational method**

We employ moving mesh method [34] to numerically solve the free boundary problem for the tumor proliferation model. To illustrate this method, we take Eq (2) as example and rewrite it as the following form:
\[
\frac{\partial D(r, t)}{\partial t} = \delta_r \Delta D(r, t) - div(uD) + F,
\]
where \( F \) represents the term in the right hand side of Eq (2). Let \( r^i_t \) and \( D^i_t \) denote numerical approximations of i-th grid point and \( D(r^i_t, n\tau) \), respectively, where \( \tau \) is the size of time-step.
The discretization of Eq (24) is derived by the fully implicit finite difference scheme:

\[
\frac{D^{k+1}_i - D^k_i}{\tau} = \delta_D \left( D_r + \frac{2}{r^k} D_s \right) - \left( \frac{2}{r^{k+1}_i} u^{k+1}_i + u_s \right) D^{k+1}_i - u^{k+1}_s D_s + F^{k+1}_i,
\]

(25)

where \( D_r = \frac{h^2_i r^{k+1}_i - h^2_i r^{k+1}_{i-1} - (h^2_i - h^2_{i-1}) r^{k+1}_i}{h_i (h^2_i - h^2_{i-1})} \), \( D_s = \frac{2 h_{i+1} r^{k+1}_{i+1} - h_i r^{k+1}_{i+1} + h_{i-1} - h_{i-2} r^{k+1}_i}{h_i (h_i - h_{i-1})} \),

\[ u_i = \frac{k_j^2 i_{j+1} - k_j^2 i_{j-1} - (h_j^2 - h_{j-1}^2) i_{j+1}}{h_j (h_j^2 - h_{j-1}^2)} \text{ and } h_i = r^{k+1}_i - r^{k+1}_{i-1} \text{ and } h_i = r^{k+1}_i - r^{k+1}_{i-1}. \]

The mesh moves by \( r^{k+1}_i = r^k_i + u^{k+1}_i \tau \), where \( u^{k+1}_i \) is solved by the velocity equation.

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