Mathematical Modeling of Interleukin-35 Promoting Tumor Growth and Angiogenesis

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Abstract

Interleukin-35 (IL-35), a cytokine from the Interleukin-12 cytokine family, has been considered as an anti-inflammatory cytokine which promotes tumor progression and tumor immune evasion. It has also been demonstrated that IL-35 is secreted by regulatory T cells. Recent mouse experiments have shown that IL-35 produced by cancer cells promotes tumor growth via enhancing myeloid cell accumulation and angiogenesis, and reducing the infiltration of activated CD8⁺ T cells into tumor microenvironment. In the present paper we develop a mathematical model based on these experimental results. We include in the model an anti-IL-35 drug as treatment. The extended model (with drug) is used to design protocols of anti-IL-35 injections for treatment of cancer. We find that with a fixed total amount of drug, continuous injection has better efficacy than intermittent injections in reducing the tumor load while the treatment is ongoing. We also find that the percentage of tumor reduction under anti-IL-35 treatment improves when the production of IL-35 by cancer is increased.

Introduction

Interleukin-35 (IL-35) is a member of the IL-12 cytokine family. It is produced in human cancer tissues such as in melanoma, B cell lymphoma [1], lung cancer, colon cancer, esophageal carcinoma, hepatocellular carcinoma, cervical carcinoma, and colorectal cancer [2,3], and it plays important roles in tumor progression and tumor immune evasion [1]. Fox3⁺ regulatory T cells (Treg) are common in tumor microenvironment [4,5], where they induce immune-suppression. They do so by producing various cytokines, including TGF-β, IL-10 [6], and IL-9 [7], thereby promoting tumor growth. It was also shown that Treg secrete IL-35 [8-14]. IL-35 functions through IL-35R on various cell types, and is a potent immune-suppressor. Indeed, Treg-derived IL-35 was shown to inhibit antitumor T cell response [15], whereas IL-35-deficient Treg have significantly reduced activity to inhibit antitumor T cell response [15]. IL-35 inhibits tumor growth via enhancing myeloid cell accumulation and angiogenesis, and reducing the infiltration of activated CD8⁺ T cells into tumor microenvironment. In the present paper we develop a mathematical model based on these experimental results. We include in the model an anti-IL-35 drug as treatment. The extended model (with drug) is used to design protocols of anti-IL-35 injections for treatment of cancer. We find that with a fixed total amount of drug, continuous injection has better efficacy than intermittent injections in reducing the tumor load while the treatment is ongoing. We also find that the percentage of tumor reduction under anti-IL-35 treatment improves when the production of IL-35 by cancer is increased.

These experimental results suggest that blocking IL-35 may be an effective therapeutic approach to human cancer. To explore this possibility we develop in the present paper a mathematical model and then conduct in silico experiments to evaluate to what extend blocking IL-35 reduces tumor growth. The model consists of a system of partial differential equations (PDEs) that involve interactions among cells (tumor cells, MDSCs, T cells, Treg, endothelial cells) and cytokines (M-CSF, TGF-β, VEGF, IL-35). We first consider the situation which corresponds to experiments in Wang et al. [1]. In these experiments two kinds of plasmacytoma cells were injected into wild type mice: tumor cells that have been transfected with IL-35 [IL58-IL-35] so that tumor secretes high amount of IL-35 into the microenvironment, and “normal” plasmacytoma cells [IL58-Ctrl] that secrete very small amount of IL-35. There is also a small amount of IL-35 produced by MDSC [17,18] as well as IL-35 produced by Treg [8-14]. We show that the model simulations agree with the experimental data in [1]. We also introduce, in this model, the effect of a drug which inhibits production of IL-35, and simulate various protocols for administering the drug. We find, that administering the drug frequently in small amounts yields better results than administering it infrequently in larger amounts. We also find that the percentage of tumor reduction under anti-IL-35 drug improves when the production of IL-35 by cancer is increased.

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Results

Mathematical model

The mathematical model is based on the network schematically shown in Figure 1. Cancer cells secrete M-CSF which attracts MDSCs; cancer cells and MDSCs secrete VEGF which triggers angiogenesis by attracting endothelial cells and enhancing their proliferation. The additional roles of MDSC are described in the caption of Figure 1. In particular, MDSC, inhibits the activation CD8+ T cells via IL-10 and a variety of other mechanisms.

As mentioned in the Introduction, Wang et al. [1] considered two kinds of tumor cells injected into mice: J558-IL-35 and J558-Ctrl. In the case of J558-IL-35, IL-35 is produced mostly by tumor cells, less by Treg, and little by MDSC. In the case of J558-Ctrl, cancer cells produce very small amount of IL-35 so that IL-35 mainly comes from Treg and MDSC. MDSC secretes TGF-β and IL-10 which promote Treg [19,20], and there is a positive feedback loop

\[ T_{\text{reg}} \rightarrow \text{IL-35} \rightarrow \text{MDSC} \rightarrow T_{\text{reg}}, \]

where the last activation is activated by TGF-β and IL-10.

We use the network described in Figure 1 to construct a system of partial differential equations. In order to simplify the computations we assume that the tumor and all the variables are radially symmetric. The variables of the model and their dimension are listed below.

- \( c(r,t) \) : tumor cell density, \( \text{cell/cm}^3 \)
- \( q(r,t) \) : M-CSF concentration, \( \text{pg/cm}^3 \)
- \( M(r,t) \) : Myeloid derived suppressor cell (MDSC) density, \( \text{cell/cm}^3 \)
- \( I_{35}(r,t) \) : Interleukin – 35 concentration, \( \text{pg/cm}^3 \)
- \( R(r,t) \) : regulatory T cell density, \( \text{cell/cm}^3 \)
- \( I_{\beta}(r,t) \) : TGF-β concentration, \( \text{pg/cm}^3 \)
- \( T(r,t) \) : T cell density, \( \text{cell/cm}^3 \)
- \( h(r,t) \) : VEGF concentration, \( \text{pg/cm}^3 \)
- \( e(r,t) \) : endothelial cell (EC) density, \( \text{cell/cm}^3 \)
- \( w(r,t) \) : oxygen concentration, \( \text{pg/cm}^3 \)

We proceed to write down the differential equation of each of the variables. Most of the parameters are taken from the literatures, as indicated; in Methods we explain how the remaining parameters were estimated.

**Tumor cell** (c). The density \( c(r,t) \) of tumor cells satisfies the following equation:
The parameters in Equation (1) are listed in Table 1. The first term on the right-hand side is the diffusion of M-CSF (the third and fourth terms) [23–25]. It was reported in [1], that MDSCs do not undergo chemotaxis driven by M-CSF, and MDSCs undergo dispersion as well as chemotaxis driven by M-CSF (the third and fourth terms) [23–25].

The first term on the right-hand side of Equation (3) represents the death of tumor cells by necrosis and apoptosis, respectively. The last term represents the killing of tumor cells by T cells [23].

The equation for the concentration of IL-35 is the following:

\[
\frac{dI_{35}}{dt} = D_{I_{35}} \frac{\partial}{\partial r} \left( r^2 \frac{\partial I_{35}}{\partial r} \right) + x_{I_{35}} c - \mu_{I_{35}} R.
\]

Experiments indicate that IL-35 can be produced by Treg [8–14]. IL-35 possesses EBI3 and IL-12p35 subunits [1,11,13,14,27]. In human model, it has been shown that EBI3 was expressed in tumor infiltrating dendritic cells [17,18], which is a subpopulation of MDSCs, and in lung cancer cells [2,3,16], whereas IL-12p35 was detected in EBI3+ tumor cells [17,18]. Hence, cancer cells

### Table 1. Parameters for the tumor cell equation.

| Parameter | Description | Dimensional | Reference |
|-----------|-------------|-------------|-----------|
| \(D_c\) | Diffusion coefficient of tumor cells | \(4.32 \times 10^{-6} \text{ cm}^2/\text{day}\) | [22,25] & estimated |
| \(c^*\) | Carrying capacity of tumor cells | \(10^6 \text{ cell/cm}^3\) | [22,47,55] |
| \(\mu_c\) | Apoptosis rate of tumor cell | \(4.15 \times 10^{-3}/\text{day}\) | [22,66] |
| \(\eta_t\) | Killing rate of tumor cells from T cells | \(3.1574 \times 10^{-6} \text{ cell/day}\) | [55,56] & estimated |
| \(\lambda_1\) | Maximal proliferation rate of tumor cells | \(2.5/\text{day}\) | [22,25,67] & estimated |
| \(\lambda_2\) | Maximal necrosis rate of tumor cells | \(8.3 \times 10^{-1}/\text{day}\) | [22,25,55,67] |
| \(w_h\) | Lower bound of oxygen in necrotic | \(3.57 \times 10^8 \text{ pg/cm}^3\) | [22,68] |
| \(w_n\) | Lower bound of oxygen in extremely hypoxic | \(10^6 \text{ pg/cm}^3\) | [22,53,68] |
| \(w_0\) | Normal oxygen level | \(4.65 \times 10^5 \text{ pg/cm}^3\) | [22,68] |
and MDSCs could be other sources of IL-35 in human and mouse cancer. Accordingly, we include the production of IL-35 by cancer cells (the second term), $T_{\text{reg}}$ (the third term), and MDSCs (the fourth term). For J558-IL-35 mouse model, we take $\gamma_{35}$ large enough and $\gamma_{35}$ small enough such that, in our simulations, $\gamma_{35}$ is relatively much larger than $\beta_{35} R$, and $\gamma_{35} M$ is significantly smaller than $\beta_{35} R$. On the other hand, in the J558-Cml mouse model, we modify $\gamma_{35}$ to be a much smaller than the value in J558-IL-35 case so that the production of IL-35 by tumor cells is significantly smaller than the productions of IL-35 by $T_{\text{reg}}$ and MDSCs. The parameters in Equation (4) are listed in Table 4.

**Regulatory T cell (R).** The equation for the density of regulatory T cells is given by

$$\frac{\partial R}{\partial t} = D_R \frac{\partial^2 R}{\partial r^2} + \frac{\delta M}{M + \sigma_R} \frac{M}{(\text{indirect}) \text{activation by MDSC}} - \delta R = \frac{I_{\beta}}{I_{\beta} + \sigma_R} - \mu_R R.$$

$T_{\text{reg}}$ is activated by TGF-$\beta$ (the third term on the right-hand side) and by IL-10. IL-10 is secreted by MDSC [19,20] and, for simplicity, we do not introduce IL-10 explicitly, and represent the activation of $T_{\text{reg}}$ by IL-10 by the term $\frac{\delta M}{M + \sigma_R}$. The parameters in Equation (5) are listed in Table 5.

**TGF-$\beta$ ($I_{\beta}$).** The equation for the concentration of TGF-$\beta$ is the following:

$$\frac{\partial I_{\beta}}{\partial t} = D_{I_{\beta}} \frac{\partial^2 I_{\beta}}{\partial r^2} + \frac{\nu_{C}}{C} + \frac{\nu_{R}}{R} - \frac{\mu_{R} I_{\beta}}{C}.$$

TGF-$\beta$ is secreted by tumor cells (second term) [28–35] and $T_{\text{reg}}$ (third term) [36–38]. The parameters in Equation (6) are shown in Table 6.

**Activated CD8$^+$ T cell (T).** Cytotoxic T cells (CTL), or CD8$^+$ T cells, satisfy the equation:

$$\frac{\partial T}{\partial t} = D_T \frac{\partial^2 T}{\partial r^2} + \frac{\delta M}{M + \sigma_R} \frac{M}{(\text{indirect}) \text{activation by IL-12}} - \frac{1}{C} \frac{\partial (a_3 M)}{\partial r} - \frac{\beta_2(a_3 M)}{(a_3 M) + r_3} \frac{M}{2} \frac{\partial I_{\beta}}{\partial r} - \mu_T T.$$

MDSC secretes MCP-1 which exerts chemotactic force on macrophages [39,40], while macrophages secrete IL-12 which

### Table 2. Parameters for the M-CSF equation.

| Parameter | Description | Dimensional | Reference |
|-----------|-------------|-------------|-----------|
| $D_M$     | Diffusion coefficient of M-CSF | $1.728 \times 10^{-1}$ cm$^2$/day | [22,25,55,67,70] |
| $\alpha_M$ | Production rate of M-CSF by tumor cell | $2.7648 \times 10^{-5}$ pg/cell/day | [22,55,71,72] |
| $\rho_M$  | Decay rate of M-CSF | $4.1472$/day | [22,73] |

### Table 3. Parameters for the MDSC equation.

| Parameter | Description | Dimensional | Reference |
|-----------|-------------|-------------|-----------|
| $\sigma_0$ | Source of MDSC | $1.10345 \times 10^5$ cell/cm$^3$/day | [56,58] & estimated |
| $\sigma_1$ | Maximal production rate via I$_{35}$ | $6.55518 \times 10^5$/day | [1] & estimated |
| $\sigma_M$ | Diffusion coefficient of MDSC | $10^5$ pg/cm$^3$/day | estimated |
| $\alpha_M$ | Diffusion coefficient of MDSC | $4.32 \times 10^{-6}$ cm$^2$/day | [22,25,26] & estimated |
| $k_M$ | Chemotaxis rate of MDSC for M-CSF | $5.2 \times 10^{-7}$ cm$^3$/pg/day | [25,55] |
| $\tau_M$ | Polarization rate of MDSC | $7.5 \times 10^{-1}$/day | [56] |
| $M_0$ | Density of myeloid precursor cells | $8 \times 10^3$ cell/cm$^3$ | [56,58] |
| $\rho_M$ | Density of myeloid precursor cells | $7.5 \times 10$ pg/cm$^3$ | [56,58] |
| $\rho_M$ | Decay rate of MDSC | $3 \times 10^{-2}$/day | [58,59] |
activates CD4+ T cells [41] and CD4+ T cells produce IL-2 [42,43] which activates CD8+ T cells. The activation of CD8+ T cells is inhibited by TGF-β [44–46]. For simplicity we combine all these process by attributing the chemotactic force or CD8+ T cells and activation source of CD8+ T cells to MDSC (the terms in square brackets in Equation (7)). The factor $s_M(\phi_M + a_1 M)$ represents the fact that MDSC suppresses CD8+ T cells proliferation by amino acid metabolism. The parameters in Equation (7) are listed in Table 7.

**VEGF (h).** The concentration of VEGF evolves according to the equation

$$\frac{\partial h}{\partial t} = D_h \frac{\partial}{\partial r} \left( r \frac{\partial h}{\partial r} \right) + \lambda_2(w) c x \frac{I_{35} + k_1}{I_{35} + \sigma_9} + \lambda_3(w) M \frac{q + k_2}{q + q_0} - \mu_s h,$$

where $\lambda_2(w) = \lambda_3 \phi(w)$ and $\lambda_3 = \lambda_\phi \phi(w)$ depend on the oxygen concentration w, as follows:

$$\phi(w) = \begin{cases} 0 & \text{if } w < w_h, \\ \exp(10(w - w_h)) - 1 & \text{if } w_h \leq w < w^*, \\ 1 - 0.7(w - w^*)/(w_0 - w^*) & \text{if } w^* \leq w \leq w_0, \\ 0.3 & \text{if } w > w_0, \end{cases}$$

and $w^*(w_h, w_0)$ is the threshold at which the hypoxic effect on VEGF production by tumor cells and MDSCs is maximal. The function $\phi(w)$ is chosen such that tumor cells and MDSCs can secrete VEGF under mild hypoxic conditions. The second term on the right-hand side of Equation (8) represents the VEGF produced by tumor cells and enhanced by $I_{35}$ [1], and the third term accounts for VEGF produced by MDSCs and enhanced by M-CSF [47]; accordingly, the ratios $k_1/\sigma_9$ and $k_2/q_0$ should be small. The parameters in Equation (8) are listed in Table 8.

**Endothelial cell (EC) (e).** The equation of the density of EC includes dispersion, chemotaxis by VEGF, and proliferation by VEGF:

$$\frac{\partial e}{\partial t} = D_e \frac{\partial}{\partial r} \left( r \frac{\partial e}{\partial r} \right) + \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial h}{\partial r} \right) + \lambda_1(1 - \frac{e}{e_1}) \frac{h - h_1}{h_0} H(h - h_1).$$

Here $e_1$ is the maximal density of EC inside the tumor, and $H(\cdot)$ is defined by

$$H(h - h_1) = \begin{cases} 1 & \text{if } h \geq h_1, \\ 0 & \text{if } h < h_1. \end{cases}$$

The last term, taken from [22], reflects the fact that VEGF induces proliferation of EC when the concentration of VEGF is higher than the threshold $h_1$. The parameters in Equation (9) are given in Table 9.

**Oxygen (w).** We model the concentration of oxygen by the equation:

| Parameter | Description | Dimensional | Reference |
|-----------|-------------|-------------|-----------|
| $D_h$     | Diffusion coefficient of $I_{35}$ | $1.25 \times 10^{-3}$ cm$^2$/day | [60] & estimated |
| $\sigma_9$ | Production rate of $I_{35}$ from tumor | $10^{-5}$ pg/cell/day for J558-IL-35 mouse | [1,16–18] & estimated |
| $s_9$     | Production rate of $I_{35}$ from tumor | $10^{-6}$ pg/cell/day for J558-Ctrl mouse | [1] & estimated |
| $h_9$     | Production rate of $I_{35}$ from Treg | $1.67 \times 10^{-3}$ pg/cell/day | [34] & estimated |
| $\gamma_9$ | Production rate of $I_{35}$ from MDSC | $10^{-4}$ pg/cell/day | [17,18] & estimated |
| $\mu_9$   | Decay rate of $I_{35}$ | 2/day | [61–63] & estimated |

Table 4. Parameters for the IL-35 equation.

Table 5. Parameters for the Treg equation.

| Parameter | Description | Dimensional | Reference |
|-----------|-------------|-------------|-----------|
| $D_{t_{reg}}$ | Diffusion coefficient of $I_{35}$ | $4.32 \times 10^{-4}$ cm$^2$/day | [22,25] & estimated |
| $\phi_{t_{reg}}$ | Maximal activation rate of $I_{35}$ by MDSC | $1.25 \times 10^6$ cell/cm$^3$/day | estimated |
| $\sigma_{t_{reg}}$ | Rate of Treg production | $10^7$ cell/cm$^3$ | estimated |
| $\phi_{t_{reg}}$ | Maximal activation rate of Treg by TGF-β | $3.327 \times 10^6$ cell/cm$^3$/day | [38] & estimated |
| $\sigma_{t_{reg}}$ | Maximal rate of Treg production | $2.4 \times 10^7$ pg/cm$^3$ | [38,64] & estimated |
| $\mu_{t_{reg}}$ | Decay rate of Treg | $10^{-1}$/day | [34,74,75] |

Table 4. Parameters for the Treg equation.

Table 5. Parameters for the Treg equation.
where the constant with a positive parameter \( \lambda \) are listed in Table 10.

Equation (10) are listed in Table 10.

We assume that the MDSC is higher at the center and negligible near the boundary.

We assume that the tumor is radially symmetric and is contained in a sphere \( 0 \leq r \leq L \), where \( L = 1.5 \text{ cm} \).

We next introduce the initial and boundary conditions for each of the variables.

**Initial conditions.** We assume that the tumor cells are concentrated initially near \( r = 0 \), and take

\[
\hat{c}(r,0) = \begin{cases} 
  c_0(e^{-r/\epsilon} - e^{-L_0/\epsilon}) & \text{if } 0 \leq r \leq L_0 \\
  0 & \text{if } L_0 < r \leq L,
\end{cases}
\]

(11)

with a positive parameter \( \epsilon \), \( 0 < \epsilon \leq 1 \), and scaling parameters \( c_0 = 7.2 \times 10^3 \text{ cell/cm}^3 \) and \( L_0 = 0.5 \text{ cm} \). Since M-CSF is secreted by tumor cells, we take the initial concentration of M-CSF to be similar to the density of tumor cells,

\[
g(0,0) = \begin{cases} 
  \frac{2q}{\mu_q}c_0(e^{-r/\epsilon} - e^{-L_0/\epsilon}) & \text{if } 0 \leq r \leq L_0 \\
  0 & \text{if } L_0 < r \leq L,
\end{cases}
\]

where the constant \( 2q/\mu_q \) comes from the steady state equation for \( q \).

Since tumor cells are concentrated at the center \( r = 0 \), we assume that the MDSC is higher at the center and negligible near the boundary \( r = L \),

\[
M(r,0) = \begin{cases} 
  \frac{\sigma_M}{\mu_M}(e^{-r/\epsilon} - e^{-L_0/\epsilon}) & \text{if } 0 \leq r \leq L_0 \\
  0 & \text{if } L_0 < r \leq L,
\end{cases}
\]

where the constant \( \sigma_M/\mu_M \) comes from the steady state equation of Equation (3). We assume that initially there are no activated CD8\(^+\) T cells, and take

\[
T(r,0) = 0 \text{ if } 0 \leq r \leq L.
\]

The activation of T\(_{reg}\)'s and the productions of I\(_{35}\) and VEGF are triggered by tumor cells and MDSCs; accordingly, we take

\[
R(r,0) = \begin{cases} 
  \frac{\delta_M + \delta_{\beta}}{\mu_R}(e^{-r/\epsilon} - e^{-L_0/\epsilon}) & \text{if } 0 \leq r \leq L_0 \\
  0 & \text{if } L_0 < r \leq L,
\end{cases}
\]

\[
I_{35}(r,0) = \begin{cases} 
  I_{35}^0(e^{-r/\epsilon} - e^{-L_0/\epsilon}) & \text{if } 0 \leq r \leq L_0 \\
  0 & \text{if } L_0 < r \leq L,
\end{cases}
\]

\[
h(r,0) = \begin{cases} 
  h_0(e^{-r/\epsilon} - e^{-L_0/\epsilon}) & \text{if } 0 \leq r \leq L_0 \\
  0 & \text{if } L_0 < r \leq L,
\end{cases}
\]

and \( I_{35}^0 = 10^2 \text{ pg/cm}^3 \), and \( h_0 = 10^3 \text{ pg/cm}^3 \). Similarly, \( I_{\beta} \) is produced by tumor cells and T\(_{reg}\)'s, so accordingly we take

\[
I_{\beta}(r,0) = \begin{cases} 
  I_{\beta}^0(e^{-r/\epsilon} - e^{-L_0/\epsilon}) & \text{if } 0 \leq r \leq L_0 \\
  0 & \text{if } L_0 < r \leq L,
\end{cases}
\]

where \( I_{\beta}^0 = 2.4 \times 10^3 \text{ pg/cm}^3 \).

Endothelial cells migrate into the tumor from the surrounding normal healthy tissue, so we take

\[
e(r,0) = \begin{cases} 
  e_0(e^{-r/\epsilon} - e^{-L_0/\epsilon}) & \text{if } 0 \leq r \leq L_0 \\
  e_0 & \text{if } L_0 < r \leq L,
\end{cases}
\]

where \( e_0 \) is the density of endothelial cell in normal healthy tissue. Finally, since endothelial cells represent capillaries through which oxygen is delivered, we prescribe

\[
w(r,0) = \begin{cases} 
  w_0(e^{-r/\epsilon} - e^{-L_0/\epsilon}) & \text{if } 0 \leq r \leq L_0 \\
  w_0 & \text{if } L_0 < r \leq L,
\end{cases}
\]

where \( w_0 \) is the oxygen concentration in normal healthy tissue.

**Boundary conditions.** Since we assume radial symmetry, the first \( r \)-derivative of each variable vanishes at \( r = 0 \). We assume no-flux condition at \( r = L \) for all the variables except for the oxygen and endothelial cells, and we take

\[
\frac{\partial c}{\partial r}(r,0) = 0 \text{ if } 0 \leq r \leq L,
\]

\[
\frac{\partial e}{\partial r}(r,0) = 0 \text{ if } 0 \leq r \leq L,
\]

\[
\frac{\partial w}{\partial r}(r,0) = 0 \text{ if } 0 \leq r \leq L.
\]
Table 7. Parameters for the CD8\(^+\) T equation.

| Parameter | Description | Dimensional | Reference |
|-----------|-------------|-------------|-----------|
| \(D_T\)   | Diffusion coefficient of T cells | \(4.32 \times 10^{-6} \text{ cm}^2/\text{day}\) | [22,25] & estimated |
| \(r_M\)   | Chemotaxis rate of T cell from MCP-1 | \(5 \times 10^6 \text{ pg/cm}^3\) | [58,77] & estimated |
| \(\beta_1\) | Production rate of IL-10 by MDSC | \(8.64 \times 10^{-4} \text{ cm}^2/\text{pg/day}\) | [78–80] & estimated |
| \(\beta_2\) | Activation rate from IL-12 | \(2.5 \times 10^{-3} \text{ cell/cm}^3/\text{day}\) | [58,77] & estimated |
| \(\alpha_1\) | Production rate of IL-10 by MDSC | \(2 \text{ pg/cell}\) | estimated |
| \(\alpha_2\) | Chemotaxis rate of MCP-1 by MDSC | \(10^{-2} \text{ pg/cell}\) | estimated |
| \(\alpha_3\) | Production rate of IL-12 by MDSC | \(10^{-2} \text{ pg/cell}\) | estimated |
| \(c_s\)   | Production rate of IL-10 by MDSC | \(7.5 \times 10 \text{ pg/cm}^3\) | [56,77] & estimated |
| \(g_p\)   | Production rate of IL-10 by MDSC | \(2.9 \times 10^{3} \text{ pg/cm}^3\) | [34] & estimated |
| \(\mu_T\) | Death rate of T cells | \(3 \times 10^{-1} \text{/day}\) | [58,81–85] |

\[
\begin{align*}
\frac{\partial v}{\partial r} + \mu (v - w) &= 0 \quad \text{at} \quad r = L, \\
\frac{\partial e}{\partial r} + \mu (e - c_0) &= 0 \quad \text{at} \quad r = L
\end{align*}
\]

(12)

where \(\mu\) is the flux rate of EC from healthy normal tissue into the tumor microenvironment.

**Parameters nondimensionalization.** We nondimensionalize the Equations (1)–(10) by the following scaling:

\[
\begin{align*}
\tilde{r} &= r/L_0, \quad \tilde{t} = t/\tau, \\
\tilde{c} &= c/c_0, \quad \tilde{q} = q/q_0, \quad \tilde{M} = M/M_0, \quad \tilde{I}_35 = I_{35}/I_{35}^0, \quad \tilde{R} = R/R_0, \\
\tilde{T} &= T/T_0, \quad \tilde{h} = h/h_0, \quad \tilde{w} = w/w_0, \\
\{\tilde{D}_c, \tilde{D}_q, \tilde{D}_M, \tilde{D}_{I35}, \tilde{D}_R, \tilde{D}_I, \tilde{D}_{h}, \tilde{D}_c, \tilde{D}_u\}
\end{align*}
\]

**Parameters for the CD8\(^+\) T equation.

Table 8. Parameters for the VEGF equation.

| Parameter | Description | Dimensional | Reference |
|-----------|-------------|-------------|-----------|
| \(D_h\)  | Diffusion coefficient of VEGF | \(8.64 \times 10^{-1} \text{ cm}^2/\text{day}\) | [22,55,86,87] |
| \(k_1\)  | Critical value of \(I_{35}\) | \(3.7 \times 10^5 \text{ pg/cm}^3\) | estimated |
| \(q_0\) | Critical value of M-CSF | \(3.7 \times 10^5 \text{ pg/cm}^3\) | estimated |
| \(k_2\)  | Critical value of M-CSF | \(10^3 \text{ pg/cm}^3\) | [22,55] |
| \(k_3\)  | Decay rate of VEGF | \(3.7 \times 10^5 \text{ pg/cm}^3\) | estimated |
| \(\mu_h\) | Decay rate of VEGF | \(10^8 \text{ pg/cm}^3\) | [22,57] |
| \(h_6\)  | Production rate of IL-10 by MDSC | \(2.86 \times 10^{-4} \text{ pg/cell/day}\) | [22,55] & estimated |
| \(\lambda_6\) | Production rate of IL-10 by MDSC | \(1.58 \times 10^{-3} \text{ pg/cell/day}\) | [22,55] |
| \(\psi^\prime\) | Production rate of IL-10 by MDSC | \(4.185 \times 10^6 \text{ pg/cm}^3\) | [22,55] & estimated |

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Table 9. Parameters for the EC equation.

| Parameter | Description | Dimensional | Reference |
|-----------|-------------|-------------|-----------|
| $D_e$ | Diffusion coefficient of EC | $4.32 \times 10^{-6}$ cm$^2$/day | [22,25,57] & estimated |
| $k_s$ | Chemotaxis force of EC by VEGF | $4.1472 \times 10^{-7}$ cm$^3$/pg/day | [22,87] & estimated |
| $\lambda_{12}$ | Proliferation rate by VEGF | $5.83 \times 10^{-1}$/day | [88] & estimated |
| $c_1$ | Maximal density of EC inside the tumor | $7.5 \times 10^7$ cell/cm$^3$ | [22] & estimated |
| $h_0$ | Scaling parameter for VEGF | $10^3$ pg/cm$^3$ | [89] & estimated |
| $h_i$ | Threshold concentration of VEGF | $1.48 \times 10^3$ pg/cm$^3$ | [90] & estimated |

$$\begin{align*}
\{\dot{\lambda}_1(w), \dot{\lambda}_2(w), \dot{\lambda}_3(w), \dot{\lambda}_6(w)\} = & \tau \{\dot{\lambda}_1(w), \dot{\lambda}_2(w), c_0 \lambda_3(w)/h_0, M^0 \lambda_6(w)/h_0\}, \\
\{\dot{\lambda}_7, \dot{\lambda}_8, \dot{\lambda}_9, \dot{\lambda}_{10}, \dot{\lambda}_{11}, \dot{\lambda}_{12}\} = & \tau \{c_0 \lambda_7/w_0, T_0 \lambda_8, M^0 \lambda_9, R_0 \lambda_{10}, c_0 \lambda_{11}, \lambda_{12}\}, \\
\sigma_0 = & \tau \sigma_0/M^0, \sigma_1 = \tau \sigma_1, \sigma_3 = \sigma_3/I_{35}^0, \sigma_M = \sigma_M/q_0, \sigma_R = \\
& \sigma_R/M^0, \sigma_B = \sigma_B/I_B^0, \\
\beta_1 = & M^0 \beta_1/L_0^2, \beta_2 = \tau \beta_2/T_0, \gamma_{35} = \tau M^0 \gamma_{35}/I_{35}^0, \eta_c = T_0 \tau_\eta_c, \\
\tilde{a}_1 = & 1, \tilde{a}_2 = 1, \tilde{a}_3 = 0.01, \tilde{e}^* = e^*/c_0, \tilde{e}_3 = c_3/M^0, \tilde{e}_M = c_M/I_{35}^0, \\
\dot{M}_0 = & 1, \\
\dot{k}_1 = & k_1/L_{35}^0, \dot{k}_2 = k_2/q_0, \dot{e}_1 = e_1/e_0, \dot{h}_1 = h_1/h_0, \dot{s}_M = s_M/M^0, \\
\dot{s}_B = & s_B/I_B^0, \\
\end{align*}$$

$c_0 = 7.2 \times 10^8$ cell/cm$^3$, $T_0 = R_0 = 10^5$ cell/cm$^3$, $M^0 = 2 \times 10^8$ cell/cm$^3$,

$e_0 = 2.5 \times 10^6$ cell/cm$^3$, $w_0 = 4.65 \times 10^3$ pg/cm$^3$,

$q_0 = h_0 = 10^3$ pg/cm$^3$, $I_{35}^0 = 10^2$ pg/cm$^3$, $I_B^0 = 2.4 \times 10^3$ pg/cm$^3$.

The dimensional and nondimensional form of the model equations in the nondimensional form are as follows:

Numerical simulation

In accordance with the experiments in Wang et al. [1], we consider two types of mice plasmacytoma J558 cells in wild type mice:

(\(\bullet\)) J558-Ctrl tumor cells that secrete a very small amount of $I_{35}$

(\(\bullet\)) J558-IL-35 tumor cells that secrete a large amount of $I_{35}$

We use matlab with $dr = 1/40$ and $dt = 7/216000$ in nondimensional variables (i.e., $dr = 1/80$ cm and $dt = 7/226000$ day in dimensional variables). Figure 2 displays the spatial distributions of tumor cell density in cases (\(\bullet\))--(\(\bullet\)) at different times. We note that, in Figure 2, as time goes on, tumor cells migrate toward the boundary $r = 1.5$ cm, where oxygen is rich while tumor cell density is lower near the center $r = 0$ cm, where oxygen is sparse. The migration speeds of these two cases (\(\bullet\))--(\(\bullet\)) are similar to each other, but tumor cells with larger $I_{35}$ production (i.e., J558-IL-35 case) have higher peak during migration.

Table 10. Parameters for the oxygen equation.

| Parameter | Description | Dimensional | Reference |
|-----------|-------------|-------------|-----------|
| $k_7$ | Delivery rate of oxygen | $6.3936 \times 10^7$ pg/cell/day | [55] |
| $D_o$ | Diffusion coefficient of oxygen | $4.32 \times 10^{-2}$ cm$^2$/day | [25,55,69,87] |
| $k_8$ | Consumption rate by T cells | $1.61568 \times 10^{-6}$ cm$^3$/cell/day | [55,65] & estimated |
| $k_9$ | Consumption rate by MDSC | $1.61568 \times 10^{-6}$ cm$^3$/cell/day | [55,56,65] & estimated |
| $k_{10}$ | Consumption rate by Treg | $1.61568 \times 10^{-6}$ cm$^3$/cell/day | [55,65] & estimated |
| $k_{11}$ | Consumption rate by tumor cells | $1.728 \times 10^{-8}$ cm$^3$/cell/day | [55,91,92] |
The results of Wang et al. [1] were reported 2 weeks after injection of tumor cells into mice. Hence, we compare our simulations at the end of the second week with the results in [1]. In Figure 3(C), the ratio for MDSC of J558-IL-35 to J558-Ctrl is 2, combining these results (Figures seven B, seven D, and seven E in [1]), we find that this ratio (for Treg/CD8+ T cells) is 0.54. From our Figures 3(E) and 3(H), we compute the ratio of J558-IL-35 to J558-Ctrl to be 0.56. Thus in all the above three cases we get a very good quantitative fit with the experimental results of Wang et al. [1]. Finally, from Figure 3(A), we see that for tumor cells the ratio of J558-IL-35 to J558-Ctrl is 2.4, which is somewhat less than the ratio for the tumor volume of B16-IL-35 mice to B16-Ctrl mice in Figure three F in [1], and significantly less for J558-IL-35 mice. This discrepancy may be explained by the fact that in vivo the

\[
\frac{\partial c}{\partial t} = D_e \frac{\partial}{\partial r} \left( r \frac{\partial c}{\partial r} \right) + \lambda_1(w)c(1 - \frac{c}{C_0}) - \lambda_2(w)c - \mu ec - \etaTCc
\]

\[
\frac{\partial q}{\partial t} = D_q \frac{\partial}{\partial r} \left( r \frac{\partial q}{\partial r} \right) + \frac{\alpha ec}{\eta M} - \mu q
\]

\[
\frac{\partial M}{\partial t} = \frac{\partial}{\partial r} \left( \frac{\partial M}{\partial r} \right) + \rho_0 + \frac{\alpha_1 M_0 \times I_{35}}{I_{35} + \rho_1} + D_M \frac{\partial}{\partial r} \left( r \frac{\partial M}{\partial r} \right) - \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 k_{35} M \frac{\partial q}{\partial r} \right)
\]

\[
\frac{\partial I_{35}}{\partial t} = D_{I_{35}} \frac{\partial}{\partial r} \left( r \frac{\partial I_{35}}{\partial r} \right) + \frac{\alpha_1 c}{\rho_{35} + \rho_{35}} + \beta_{35} R + \gamma_{35} M - \mu_{35} I_{35}
\]

\[
\frac{\partial R}{\partial t} = D_R \frac{\partial}{\partial r} \left( r \frac{\partial R}{\partial r} \right) + \frac{\delta M M}{M + \sigma_R} + \frac{\delta R I_{35}}{I_{35} + \sigma_R} - \mu_{35} R
\]

\[
\frac{\partial I_{35}}{\partial t} = D_{I_{35}} \frac{\partial}{\partial r} \left( r \frac{\partial I_{35}}{\partial r} \right) + \frac{\alpha_1 c}{\rho_{35} + \rho_{35}} + \beta_{35} R + \gamma_{35} M - \mu_{35} I_{35}
\]

which is the same as Figure five A in [1]. In Figure 3(H), the ratio for VEGF of J558-IL-35 to J558-Ctrl is 17, which is the approximately same as Figure four D in [1]. Next, we compare the ratio for Treg/CD8+ T cells of J558-IL-35 to J558-Ctrl with the result in [1]. But, in [1], they only showed the percentages of CD8+/CD45+, of CD4+/CD45+, and of Foxp3+/CD4+. By
### Table 11. Model parameters and units.

| Parameter | Dimensional | Dimensionless |
|-----------|-------------|---------------|
| $D_1$     | $4.32 \times 10^{-6}$ cm$^2$/day | $5.184 \times 10^{-5}$ |
| $D_2$     | $1.728 \times 10^{-1}$ cm$^2$/day | 2.073 |
| $D_M$     | $4.32 \times 10^{-6}$ cm$^2$/day | $5.184 \times 10^{-5}$ |
| $D_L$     | $1.25 \times 10^{-3}$ cm$^2$/day | $1.5 \times 10^{-2}$ |
| $D_R$     | $4.32 \times 10^{-6}$ cm$^2$/day | $5.184 \times 10^{-5}$ |
| $D_T$     | $8.64 \times 10^{-2}$ cm$^2$/day | 1.0368 |
| $D_Y$     | $4.32 \times 10^{-6}$ cm$^2$/day | $5.184 \times 10^{-5}$ |
| $D_0$     | $8.64 \times 10^{-2}$ cm$^2$/day | 1.0368 |
| $D_s$     | $4.32 \times 10^{-6}$ cm$^2$/day | $5.184 \times 10^{-5}$ |
| $s_8$     | $2.7648 \times 10^{-5}$ pg/cell/day | $5.97197 \times 10$ |
| $s_{63}$  | $7.5 \times 10^{-1}$/day | 2.25 |
| $s_{55}$  | $10^{-3}$ pg/cell/day for J558-IL-35 mouse | $2.16 \times 10^6$ for J558-IL-35 mouse |
| $s_{15}$  | $10^{-7}$ pg/cell/day for J558-Ctrl mouse | $2.16$ for J558-Ctrl mouse |
| $b_{55}$  | $1.67 \times 10^{-3}$ pg/cell/day | 5 |
| $\gamma_{55}$ | $10^{-4}$ pg/cell/day | $6 \times 10^2$ |
| $\delta_{55}$ | $1.25 \times 10^0$ cell/cm$^3$/day | $3.75 \times 10$ |
| $\delta_s$ | $3.327 \times 10^6$ cell/cm$^3$/day | 99.81 |
| $q_L$     | $3.1574 \times 10^{-6}$ cm$^3$/cell/day | $9.47232 \times 10^{-1}$ |
| $q_0$     | $5.51725 \times 10^4$ cell/cm$^3$/day | $8.2759 \times 10^4$ |
| $q_1$     | $4.65518 \times 10^3$/day | $1.39655 \times 10^3$ |
| $q_{55}$  | $7.5 \times 10$ pg/cm$^3$ | $7.5 \times 10^{-2}$ |
| $\gamma_R$ | $10^2$ cell/cm$^3$ | $5 \times 10^{-2}$ |
| $\sigma_R$ | $2.4 \times 10^1$ pg/cm$^3$ | 1 |
| $\sigma_5$ | $3.7 \times 10^0$ pg/cm$^3$ | $3.7 \times 10^0$ |
| $\lambda_1$ | $2.5$/day | 7.5 |
| $\lambda_2$ | $8.3 \times 10^{-1}$/day | 2.49 |
| $\lambda_3$ | $2.86 \times 10^{-4}$ pg/cell/day | $6.1776 \times 10^2$ |
| $\lambda_4$ | $1.58 \times 10^{-1}$ pg/cell/day | $9.48 \times 10^2$ |
| $\lambda_5$ | $6.3936 \times 10^2$ pg/cell/day | $1.03123 \times 10$ |
| $\lambda_6$ | $1.61568 \times 10^{-8}$ cm$^3$/cell/day | $4.84704 \times 10^{-3}$ |
| $\lambda_7$ | $1.61568 \times 10^{-8}$ cm$^3$/cell/day | $9.69408$ |
| $\lambda_{10}$ | $1.61568 \times 10^{-8}$ cm$^3$/cell/day | $4.84704 \times 10^{-3}$ |
| $\lambda_{11}$ | $1.728 \times 10^{-6}$ cm$^3$/cell/day | $3.73248 \times 10$ |
| $\lambda_{12}$ | $5.83 \times 10^{-1}$/day | 1.75 |
| $\nu_c$   | $5.5 \times 10^{-6}$ pg/cell/day | 4.95 |
| $\nu_R$   | $9 \times 10^{-7}$ pg/cell/day | $1.125 \times 10^{-4}$ |
| $\nu$     | 1 | 1 |
| $\nu_5$   | $4.15 \times 10^{-1}$/day | 1.245 |
| $\nu_4$   | $4.1472$/day | $1.24416 \times 10$ |
| $\nu_M$   | $3 \times 10^{-2}$/day | $9 \times 10^{-2}$ |
| $\nu_{55}$ | 2/day | 6 |
| $\nu_{R}$ | $10^{-1}$/day | $3 \times 10^{-1}$ |
| $\nu_5$   | $0.693$/day | 2.079 |
| $\nu_Y$   | $3 \times 10^{-1}$/day | $9 \times 10^{-1}$ |
| $\nu_6$   | $1.08864 \times 10$/day | $3.26592 \times 10$ |
| $\mu$     | 10/cm | 5 |
arrival of MDSCs to the tumor microenvironment is somewhat delayed and therefore the number of CD8+ T cells in the control case is significantly less than in the J558-IL-35 case, while (for simplicity) our model does not include such a time delay.

The subunits of IL-35, EBI3 and IL-12p35, are highly expressed in cancers such as lung cancer, colorectal cancer, and esophageal carcinoma [2,3]. Anti-IL-35 drug blocks the expression of IL-35 and could be an agent in treating these cancers [48]. To determine the effect of anti-IL-35 drug on cancer growth, we proceed to introduce it, as a drug, into our model. If we denote its concentration by \( f(r,t) \) then all we need to do is to modify Equation (4) by

\[
\frac{\partial I_{35}}{\partial t} = D_{I_{35}} \frac{\partial}{\partial r} \left( \frac{\partial I_{35}}{\partial r} \right) + \frac{1}{f(r,t)} \left[ \gamma_{I_{35}} C_{35} + \beta_{I_{35}} R + \gamma_{I_{35}} M - \mu_{I_{35}} I_{35} \right].
\]
Figure 2. Spatial distributions of tumor cells. (A), (B), (C), and (D) are the spatial distributions of tumor cells \( c(r,t) \) in the mice model at the end of the 2nd, 4th, 6th, and 8th weeks, respectively, for cases (i) and (ii). The thin curve is the initial value of tumor cells for the cases (i) and (ii). The solid curve is for J558-IL-35 tumor cells with large \( I_{35} \) production (case (ii)) and the dashed curve is for J558-Ctrl tumor cells (case (i)).

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Figure 3. Evolution of cells and cytokines for J558-IL-35 and J558-Ctrl mice models. Panels (A) to (J) show the profiles of the total numbers of tumor cells, M-CSF, MDSCs, \( I_{35} \), T\(_{reg}\), TGF-\( \beta \), CD8\(^+\) T cells, VEGF, endothelial cells, and oxygen, for cases (i) and (ii). The solid curve is for J558-IL-35 tumor cells with large \( I_{35} \) production (case (ii)) and the dashed curve is for J558-Ctrl tumor cells (case (i)).

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We make the pharmacokinetic assumption that \( f(r,t) \) decreases in \( r \) from the outer boundary of the tumor \( (r = 0) \) towards the center of the tumor \( (r = \text{r center of the tumor}) \), and take

\[
f(r,t) = F \times \frac{r^2 + a}{L^2 + a},
\]

where \( a = L^2 (\approx 2.25 \text{ cm}^2) \) and \( F = 10 \). We shall compare several dosing schedules:

(i) no dosing of anti-IL-35, i.e., \( f(r,t) = 1 \), for all \( t \) and \( 0 \leq r \leq L \);

(ii) continuous dosing with anti-IL-35 at fixed level \( F \) for 2 months,

\[
f(r,t) = F \times \frac{r^2 + a}{L^2 + a}, \quad \text{for } 0 \leq r \leq L \text{ and } 0 \leq t \leq 2 \text{ months};
\]

(iii) intermittent dosing for 2 months, at double level \( 2F \), one week at a time with one week spacing between dosing,

\[
f(r,t) = \begin{cases} 
2F \times \frac{r^2 + a}{L^2 + a}, & \text{for } 0 \leq r \leq L \text{ and } t_2i \leq t < t_{2i+1}, \\
0, & \text{for } 0 \leq r \leq L \text{ and } t_{2i+1} \leq t < t_{2(i+1)},
\end{cases}
\]

for \( i = 0, 1, 2, 3 \), where \( t_0 = 0 \) and the length of each interval \( [t_i, t_{i+1}] \) is one week.

We use matlab with \( dr = 1/80 \text{ cm} \) and \( dt = 7/24000 \text{ day} \) in dimensional variables. Figure 4 shows that the temporal growth of the total numbers of tumor cells, as functions of time, under

(A) \( \chi_{35} = 10^{-4} \text{ pg/cell/day}; \)

(B) \( \chi_{35} = 5 \times 10^{-4} \text{ pg/cell/day}; \)

and (C) \( \chi_{35} = 10^{-3} \text{ pg/cell/day}. \)

Figure 4 indicates that the continuous treatment has better efficacy in reducing tumor load than intermittent treatment when \( \chi_{35} \in [10^{-4} \text{ pg/cell/day}, 10^{-3} \text{ pg/cell/day}] \). Figure 4 also shows that the reduction rate by anti-IL-35 is larger when tumor cells secrete higher amount of IL-35 as in Lung cancer and colorectal cancer [2,3] than lower amount of IL-35 as in plasmacytoma [1]. Accordingly, as \( \chi_{35} \) increases, the reduction in total tumor population becomes increasingly significant.

**Sensitivity analysis**

In this section we perform sensitivity analysis on the parameters (in dimensional form) including those that were only roughly estimated and those that play important role in the model. We list these parameters with their ranges, baselines, and units in Table 13. We use the method described in Marino et al. [49], using the Latin hypercube sampling to generated 500 samples with \( dr = 1/40 \text{ cm} \) and \( dt = 7/12000 \text{ day} \).
Table 13. Parameters chosen for sensitivity analysis.

| Parameter | Range          | Baseline | Unit          |
|-----------|----------------|----------|---------------|
| $\gamma_M$ | $[3.75 \times 10^{-1}, 1.5]$ | $7.5 \times 10^{-1}$ | /day          |
| $\delta_M$ | $[6.25 \times 10^{4}, 2.5 \times 10^{6}]$ | $1.25 \times 10^{6}$ | cell/cm$^3$/day |
| $\beta$ | $[1.6635 \times 10^{6}, 6.654 \times 10^{6}]$ | $3.327 \times 10^{4}$ | cell/cm$^3$/day |
| $\alpha$ | $[10^{-4}, 10^{-3}]$ | $5 \times 10^{-4}$ | pg/cell/day    |
| $\beta_35$ | $[8.35 \times 10^{-4}, 3.34 \times 10^{-3}]$ | $1.67 \times 10^{-3}$ | pg/cell/day    |
| $\gamma$ | $[5 \times 10^{-4}, 2 \times 10^{-4}]$ | $10^{-4}$ | pg/cell/day    |
| $r_c$ | $[2.75 \times 10^{-6}, 1.1 \times 10^{-5}]$ | $5.5 \times 10^{-6}$ | pg/cell/day    |
| $\eta$ | $[4.5 \times 10^{-1}, 1.8 \times 10^{-4}]$ | $9 \times 10^{-1}$ | pg/cell/day    |
| $\alpha_5$ | $[1.5787 \times 10^{-6}, 6.3148 \times 10^{-6}]$ | $3.1574 \times 10^{-6}$ | cm$^3$/cell/day |
| $\sigma_0$ | $[2.75863 \times 10^4, 1.10345 \times 10^5]$ | $5.51725 \times 10^4$ | cell/cm$^5$   |
| $\sigma_1$ | $[2.32759 \times 10^2, 9.31036 \times 10^2]$ | $4.65518 \times 10^2$ | /day          |
| $\sigma_2$ | $[5 \times 10^{5}, 2 \times 10^{10}]$ | $10^9$ | cell/cm$^5$   |
| $\sigma_3$ | $[1.2 \times 10^{1}, 4.8 \times 10^{1}]$ | $2.4 \times 10^{0}$ | pg/cm$^3$     |
| $\sigma_4$ | $[1.85 \times 10^{6}, 7.4 \times 10^{4}]$ | $3.7 \times 10^{0}$ | pg/cm$^3$     |
| $\kappa_M$ | $[5 \times 10^{1}, 2 \times 10^{10}]$ | $10^6$ | pg/cm$^3$     |
| $\epsilon$ | $[1.45 \times 10^{5}, 5.8 \times 10^{6}]$ | $2.9 \times 10^{1}$ | pg/cm$^3$     |
| $\kappa_M$ | $[2.5 \times 10^{3}, 10^7]$ | $5 \times 10^3$ | cell/cm$^5$   |
| $\alpha_1$ | $[1.4]$ | $2$ | pg/cell      |
| $\alpha_2$ | $[5 \times 10^{-3}, 2 \times 10^{-2}]$ | $10^{-2}$ | pg/cell      |
| $\kappa_3$ | $[5 \times 10^{-1}, 2 \times 10^{-2}]$ | $10^{-2}$ | pg/cell      |
| $\kappa_4$ | $[1.85 \times 10^{5}, 7.4 \times 10^{4}]$ | $3.7 \times 10^5$ | pg/cm$^3$     |
| $\kappa_5$ | $[5, 20]$ | $10$ | pg/cm$^3$     |
| $\kappa_6$ | $[1.25, 5]$ | $2.5$ | /day         |
| $\kappa_7$ | $[1.43 \times 10^{-4}, 5.72 \times 10^{-4}]$ | $2.86 \times 10^{-4}$ | pg/cell/day |
| $\kappa_8$ | $[7.9 \times 10^{-4}, 3.16 \times 10^{-3}]$ | $1.58 \times 10^{-3}$ | pg/cell/day |
| $\kappa_{10}$ | $[2.42352 \times 10^{-3}, 9.69408 \times 10^{-3}]$ | $4.84704 \times 10^{-3}$ | cm$^3$/cell/day |
| $\kappa_{12}$ | $[8.75 \times 10^{-4}, 3.5]$ | $1.75$ | /day         |
| $e_1$ | $[3.75 \times 10^6, 1.5 \times 10^7]$ | $7.5 \times 10^6$ | cell/cm$^5$   |
| $b_1$ | $[7 \times 10^2, 2.96 \times 10^3]$ | $1.48 \times 10^1$ | pg/cm$^3$     |

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Since we focus on how anti-IL-35 drug inhibits tumor growth, we calculate the partial rank correlation coefficients (PRCC) and p-value, corresponding to the ratio $C = \int_0^r c(r,t) r^2 dr / \int_0^r c(r,t) r^2 dr$ for $t = 2$ months, where $c(r,t)$ accounts for continuous treatment and $c(r,t)$ accounts for of no drug; $C$ is a measure of the (relative) efficacy of the drug. In this analysis, all the parameters are chosen in the range from half to twofold of their baseline, except $\gamma_35$ which is chosen from $10^{-5}$ pg/cell/day to $10^{-3}$ pg/cell/day. Table 14 lists the PRCC and their p-values. Figure 3 plots the PRCC of the parameters with p-values smaller than 0.01. A negative PRCC (i.e. negative correlation) with p-value smaller than 0.01 means that increasing this parameter value will decrease the value of $C$ and hence increase the (relative) efficacy of the drug. A positive PRCC with p-value smaller than 0.01 has the opposite meaning, that is, it will decrease the efficacy of the drug.

In Table 14, only $\eta_s$, $e_1$, $\lambda_s$, $s_M$, $s_\beta$, $\gamma_35$, and $\beta_35$ have negative PRCC with p-value smaller than 0.01. The most significant negatively correlated parameter is $\eta_s$. Larger $\lambda_s$ increases the production of VEGF and larger $\gamma_35$ increases the production of $I_{35}$ and both increase tumor load. The negative correlation of these parameters shows that the drug is more effective for tumor with higher rate of production of VEGF and IL-35. On the other hand, the negative correlation of $\eta_s$ shows that the efficacy of the drug improves when the CD8$^+$ T cells are more effective in killing tumor cells. However, it is not true to conclude that, in general, the drug efficacy increases with larger tumor load, since larger $\eta_s$ and $s_\beta$ shrink the tumor load but yield better drug efficacy. Similar results hold for the parameters with positive PRCC. For example, larger $\lambda_3$ and $s_\beta$ lead to higher tumor cell population while the tumor efficacy is decreased.

Discussion

IL-35 is the most anti-inflammatory cytokine within the IL-12 cytokine family. In this paper we addressed the questions to what extend IL-35 is involved in tumor microenvironment and how effective is anti-IL-35 drug in reducing tumor growth. It is well known that $T_{reg}^5$ are present in the tumor microenvironment.
and that they secrete IL-35 to promote tumor growth. Recent mouse experiments of Wang et al. [1] determined the extent to which IL-35 enhanced the MDSC population and the VEGF concentration, and at the same time decreased the CD8+ T cell population. Based on these experiments, we developed a mathematical model which includes in addition to tumor cells, MDSCs, CD8+ T cells, IL-35, and VEGF, also Treg, endothelial cells, oxygen concentration, TGF-β, and M-CSF that is produced by cancer cells. The model is described by a system of partial differential equations. The simulations of the model are in qualitative agreement with the experimental results of Wang et al. [1].

We next extended the model to include anti-IL-35 as an anti-cancer drug. We compared the efficacy of the drug under two schedules: continuous versus intermittent injections of the same total amount of the drug. We found that continuous injection has better efficacy while the treatment is ongoing. Since it is well known that some cancers including lung and colorectal cancers most likely secrete large amounts of IL-35, we also investigated the efficacy of the drug for such cancers. We found that the percentage of tumor reduction under anti-IL-35 drug improves when the production of IL-35 by cancer is increased.

There are currently only few experimental results by which our model can be tested. In recent experiments by Nicholl et al. [50] it was demonstrated that IL-35 promotes pancreatic cancer cells proliferation while anti-IL-35 reduces this promotion. More specifically, in Figure three of Nicholl et al. [50] it is shown that IL-35 [50 ng/ml] increases, on the average, by 100% the proliferation of colonies of several pancreatic cancer cell lines, while in the presence of anti-IL-35 (200 ng/ml) this increase is reduced to 50%. These in vitro results are in qualitative agreement with our results in Figure three (at week 8). Another example is taken from colorectal cancer in patients. As reported in Zeng et al. [2], Foxp3+ Treg increases linearly with IL-35, and this is in qualitative agreement with Figures 3D and 3E of our simulations. As more experimental and clinical data become available, we should be able to test our model in more quantitative way, so that the model can further be refined.

In this paper we focused on the role of IL-35, although Treg secrete besides IL-35 also other cytokines that promote tumor, such as IL-10 and IL-9 [7,51–54]; these were not included directly in the present model, since we wanted to base the model on the recent experimental data by Wang et al. [1]. When data for other cytokines become available to the same precision as, for instance, in [1], our model could then be extended to include these cytokines, and to obtain a more comprehensive evaluation of anti-IL-35 efficacy in combination with other drugs.

### Methods

#### Estimate $D_e$, $\eta_c$ and $\lambda_1$ in Equation (1)

We assume that the killing efficiency of tumor cells by CD8+ T cells is suppressed by IL-35 and that the proliferation rate of tumor cells is enhanced by IL-35. Accordingly in Equation (1), we choose smaller killing rate $\eta_c$ [55,56] and larger proliferation rate $\lambda_1$ of tumor cells than in [22,55]. For simplicity, we take all cells to have the same diffusion coefficient, $D_e = D_M = D_R = D_T = D_c$, with $D_c = 4.32 \times 10^{-6} \text{cm}^2/\text{day}$ by [22,25,57].

#### Estimate $c_M$ in Equation (3)

From Figures two B and three B in [1], we deduce that $I_{35}$ grows slowly in time, and

$$I_{35}(0) \approx 1.8 \times 10^5 \text{pg/cm}^3 \text{ and } I_{35}(15) \approx 5.6 \times 10^5 \text{pg/cm}^3.$$  \hspace{1cm} (18)

We take $c_M = 10^6 \text{pg/cm}^3$ so that on the average $\frac{I_{35}}{I_{35} + c_M} \approx \frac{1}{5}$ for $0 < t < 15$ days.

#### Estimate $\sigma_0$, $\sigma_1$, and $\alpha_M$ in Equation (3)

In order to estimate $\sigma_1$, we use simplified forms of Equation (3):

$$\frac{dM}{dt} = \sigma_0 + \alpha_M \times \frac{qM_0}{\sigma_M + q} - \mu_M M,$$  \hspace{1cm} (19)

$$\frac{dM}{dt} = \sigma_0 + \sigma_1 M_0 \times \frac{I_{35}}{I_{35} + c_M} + \alpha_M \times \frac{qM_0}{\sigma_M + q} - \mu_M \tilde{M},$$  \hspace{1cm} (20)

for J558-Ctrl tumor cells and J558-IL-35 tumor cells, respectively. Taking the difference and recalling that on the average $\frac{I_{35}}{I_{35} + c_M} \approx \frac{1}{5}$ for $0 < t < 15$, we get, with $\mu_M = 0.03/\text{day}$ [58,59].

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### Table 14. The PRCC and p-value of parameters for sensitivity analysis.

| Parameter | PRCC | p-value |
|-----------|------|---------|
| $\gamma_M$ | -0.0039409 | > 0.01 |
| $D_M$ | -0.040652 | > 0.01 |
| $\delta_M$ | -0.045366 | > 0.01 |
| $s_{15}$ | -0.15449 | < 0.01 |
| $\beta_{35}$ | -0.12796 | < 0.01 |
| $\tau_M$ | 0.055333 | > 0.01 |
| $\tau_c$ | 0.17422 | < 0.01 |
| $\tau_R$ | 0.021612 | > 0.01 |
| $g_c$ | -0.7056 | < 0.01 |
| $\rho_0$ | 0.22963 | < 0.01 |
| $\rho_1$ | 0.074071 | > 0.01 |
| $\rho_R$ | -0.03105 | > 0.01 |
| $\rho_3$ | 0.022536 | > 0.01 |
| $c_{M0}$ | 0.14064 | > 0.01 |
| $l_M$ | 0.12563 | > 0.01 |
| $l_y$ | -0.20223 | < 0.01 |
| $i_M$ | -0.25416 | < 0.01 |
| $i_1$ | 0.33607 | < 0.01 |
| $i_2$ | -0.0067372 | > 0.01 |
| $i_1$ | 0.014791 | > 0.01 |
| $k_1$ | 0.06582 | > 0.01 |
| $k_2$ | -0.070145 | > 0.01 |
| $v_M$ | 0.75819 | < 0.01 |
| $v_S$ | -0.26421 | < 0.01 |
| $v_S$ | -0.0097113 | > 0.01 |
| $v_{10}$ | 0.040952 | > 0.01 |
| $v_{12}$ | -0.093337 | > 0.01 |
| $c_1$ | -0.30227 | < 0.01 |
| $b_1$ | 0.28538 | < 0.01 |

...
\[
\hat{M}(15) - M(15) = (\hat{M}(0) - M(0))e^{-0.45} + \frac{\sigma_1 M_0}{\hat{m}_M}(1 - e^{-0.45})
\]

and the first term of the right-hand side may be neglected since initially the density of MDSC is small [1]. From Figure five A in [1], we deduce that

\[
\hat{M}(15) \approx 18 \times 10^6 \text{ cell/cm}^3/\text{day} \quad \text{and} \quad M(15) \approx 9 \times 10^6 \text{ cell/cm}^3/\text{day}.
\]

Since \(M_0 = 8000 \text{ cell/cm}^3 [56,58]\), we get

\[
\sigma_1 = \frac{5}{8000 \text{ cell/cm}^3} \times \frac{0.03/\text{day} \times 9 \times 10^6 \text{ cell/cm}^3}{1 - e^{-0.45}} \\
\approx 465.518/\text{day}.
\]

We assume that, due to the secretion of IL-35, the production of MDSC in the present model is larger than the production assumed in [56], so we have taken \(\sigma_0\) and \(\sigma_M\) to be larger than in [56].

**Estimate \(D_{35}\) and \(\mu_{35}\) in Equation (4)**

Since IL-35 belongs to the IL-12 family, we assume that its diffusion coefficient and its degradation rate are the same as for IL-12 [60–63]:

\[
D_{35} = 1.25 \times 10^{-3} \text{ cm}^2/\text{day}, \\
\mu_{35} = 2/\text{day}.
\]

**Estimate \(\zeta_{35}, \beta_{35}, \gamma_{35}\) in Equation (4)**

In order to find \(\zeta_{35}\) for the J558-IL-35 mouse model, we use the simplified version of Equation (4) where only cancer cells produce \(I_{35}\), i.e., \(R = 0\) and \(M = 0\):

\[
\frac{dI_{35}(t)}{dt} = \zeta_{35}c - \mu_{35}I_{35}(t).
\]

If \(c\) is taken to be a constant, then

\[
I_{35}(t) = e^{-\mu_{35}t}I_{35}(0) + \frac{\zeta_{35}c}{\mu_{35}}(1 - e^{-\mu_{35}t}).
\]

In the in vivo experiments of Wang et al. [1] the initial number of cancer cells that were injected was \(5 \times 10^6\) and we assume that they occupy a volume of 50 mm³, so that

\[
c(0) = 10^8 \text{ cell/cm}^3.
\]

There is no data in [1] on the density of the tumor cells in day 15, but the tumor cells were observed to grow rapidly in the first 15 days. We assume that the average of the density of tumor cells in the first 15 days is very close to the maximal capacity \(10^9 \text{ cell/cm}^3\) and take, in (23), \(c = 10^9 \text{ cell/cm}^3\) for J558-IL-35 tumor cells. Recalling Equation (18), we get, with \(\mu_{35} = 2/\text{day}\) (Table 4),

\[
5.6 \times 10^5 \text{ pg/cm}^3 \approx e^{-15 \text{ day} \times 2/\text{day}} \times 1.8 \times 10^5 \text{ pg/cm}^3.
\]
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Estimate $v_r$ and $v_R$ in Equation (6)

We assume as before that the initial tumor occupies a volume of 50 mm$^3$ and, accordingly, also $T_{reg}$ occupies the same volume. In [34], the production of $I_\beta$ by tumor cells and $T_{reg}$ are $1.1 \times 10^{-4} \frac{pg}{day\cdot cell} \times \frac{1}{cm^3}$ and $1.8 \times 10^{-5} \frac{pg}{day\cdot cell} \times \frac{1}{cm^3}$, respectively. Hence,

\[ v_r = 1.8 \times 10^{-5} \frac{pg}{day\cdot cell} \times \frac{1}{cm^3} \times 50 \, mm^3 = 9 \times 10^{-7} \, pg/cell/day, \]

\[ v_R = 1.1 \times 10^{-4} \frac{pg}{day\cdot cell} \times \frac{1}{cm^3} \times 50 \, mm^3 = 5.5 \times 10^{-6} \, pg/cell/day. \]

Estimate $s_M$, $\beta_1$, $\beta_2$, $a_1$, $a_2$, $a_3$, $c_5$ in Equation (7)

Since IL-35 enhances the population of MDSC, the concentration of IL-10, which we represent by $a_1 M$, is larger than the one in [56]. Hence, we chose $s_M$ to be larger than the corresponding value of $s_M$ in [56]. Moreover, since IL-35 promotes tumor growth, we expect a stronger immune response by $T$ cells than in [56] and hence we take $\beta_1$ and $\beta_2$ larger than the corresponding value in [56]. The parameter $c_5$ is taken from [56]. Since the chemotaxis and activation of CD8$^+$ T cells are indirect, we take $a_2$ and $a_3$ to be smaller than $a_1$: $a_1 = 2 \, pg/cell$ and $a_2 = a_3 = 0.01 \, pg/cell$.

Estimate $k_1$, $k_2$, $a_\beta$, $\lambda_5$, $w_4$ in Equation (8)

We take $a_\beta$ to be the average of the concentration of IL-35 at times 0 and 15 days, so that $a_\beta = 3.7 \times 10^5 \, pg/cm^3$ by Equation (18). We assume that the productions of VEGF by tumor cells and MDSCs are small when there are no IL-35 and M-CSF, respectively, so we set $k_1 = 3.7 \times 10^2 \, pg/cm^3$ and $k_2 = 10 \, pg/cm^3$. Since in [1] $I_{35}$ increases the concentration of VEGF significantly, we take $\lambda_5$ to be larger than the value in [56]. We also slightly modify the parameter value $w_4$ and function $\beta$ used in [56].

Estimate $D_e$, $k_b$, $\lambda_{12}$, $e_1$, $h_0$, and $h_1$ in Equation (9)

We take values similar to those in [22,55].

Estimate $\lambda_8$, $\lambda_9$, and $\lambda_{10}$ in Equation (10)

We assume that CD8$^+$ T cells, MDSCs, and $T_{reg}$ have the same consumption rates of oxygen, so we take $\lambda_8 = \lambda_9 = \lambda_{10} = 1.61568 \times 10^{-8} \, cm^3/cell/day$ [55,56,65].

Author Contributions

Conceived and designed the experiments: KL, XB AF. Performed the experiments: KL, XB AF. Analyzed the data: KL, XB AF. Contributed reagents/materials/analysis tools: KL, XB AF. Wrote the paper: KL, XB AF.

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