CHAPTER 4

Cancer and the immune system

A **solid tumor** is an abnormal new growth of tissue that has no physiological function. If this new growth is localized, it is called a **benign tumor**, while, if it spreads to other parts of the body, it is called a **malignant tumor**, or a **cancer**. However, people often use the words tumor and cancer interchangeably.

Cancer cells are normal cells that have been transformed by mutations in genes that regulate growth and proliferation. These genes are either **oncogenes** which promote growth and reproduction, or **suppressor genes** which inhibit cell division and survival. Tumor occurs when oncogenes become abnormally overexpressed, or when suppressor genes are disabled or become abnormally underexpressed. Tumor is believed to develop as a result of mutations in several genes, not just one.

There are more than 100 types of cancer, characterized by the organ or tissue where the cancer forms, or by the type of cells that form the cancer. In what follows we consider only solid tumors, that is, tumors that develop in a tissue, rather than in blood cells.

Tumor growth and malignancy typically induce moderate cellular response. While the immune system attempts to destroy cancer cells, cancer cells try to evade the immune response by manipulating it in different ways. The interaction between cancer cells and the immune system is currently an intensive area of research, including animal experiments and clinical trials.

The aim of clinical trials is to determine whether a specific drug will be effective in suppressing or eliminating the tumor without causing unacceptable negative side-effects. But clinical trials take several years and they can be very expensive. On the other hand, mathematical models that correctly capture the biology of the cancer and its microenvironment, can serve as "clinical trials *in silico*," and may be used to support the choice of specific drugs for clinical trials. The mathematical models can also be used to suggest optimal protocols for applying the drug; that is, how often and at what amounts should the drugs be administered.

In this chapter we introduce two mathematical models that involve tumor-immune interactions in the context of two different drugs.

In the first model the drug is TGF-β inhibitor, which is currently used for some types of cancer. The list of variables of the model is given in Table 4.1, and the units are all in g/cm^3.

Figure 4.1 is a schematics of a network showing how cells and cytokines interact with each other. The mathematical model will be represented by a system of partial differential equations based on Fig. 4.1. We note that the tumor region, $\Omega(t)$, varies in time, and its boundary $\partial\Omega$ is a "free boundary," that is, it is a boundary which is not *a priori* prescribed and needs to be determined together with the solution of the differential equations.

TABLE 1. List of variables (in unit of g/cm^3).

Notation	Description
C	density of cancer cells
D	density of dentritic cells
T_1	density of Th1 cells
T_8	density of CD8$^+$ T cells
T_r	density of T regulatory cells (Tregs)
I_{12}	concentration of IL-12
I_2	concentration of IL-2
T_β	concentration of TGF-β
I	concentration of TGF-β inhibitor

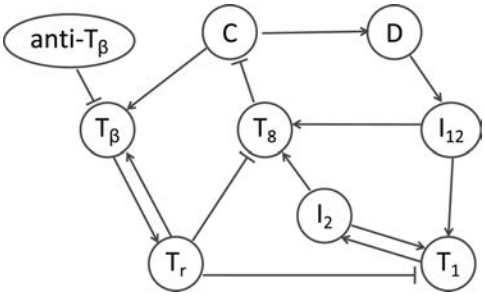

FIGURE 4.1. Network of cells and cytokines; sharp arrows represent reproduction/activation, and blocked arrows represent inhibition/killing.

We assume that the density of the cells in the tumor tissue is uniform, and take

$$C + D + T_1 + T_8 + T_r \equiv \theta \text{ g/cm}^3 \quad \text{in} \quad \Omega(t),\ t > 0, \tag{4.1}$$

where $0 < \theta \leq 1$.

Since cancer cells proliferate abnormally fast while immune cells migrate into the tumor, Eq. (4.1) implies that there is an internal pressure among the cells within $\Omega(t)$, which gives rise to velocity $\vec{u}$. We assume that all the cells are subject to the same velocity, $\vec{u}$.

As tumor grows, the cells in the inner core do not receive enough oxygen, a condition called **hypoxic**, and they undergo death by necrosis. Cells undergoing necrosis secrete cytokine HMGB-1 which is known to activate dendritic cells. The activation of the immature, or inactive, dendritic cells, D_0, is proportional to

$$D_0 \frac{\text{HMGB-1}}{K + \text{HMGB-1}}$$

for some parameter K. We assume that HMGB-1 is proportional to the density of necrotic cells, and that the density of necrotic cells is proportional to the density of cancer cells, C. Hence, the activation of dendritic cells is given by

$$\lambda_D D_0 \frac{C}{K_C + C}$$

for some positive parameters λ_D and K_C.

We recall that D cells move with velocity $\vec{u}$, and we assume that they are also subject to small dispersion, or diffusion. Hence

$$\frac{\partial D}{\partial t} + \nabla \cdot (\vec{u} D) - \delta_D \nabla^2 D = \lambda_D D_0 \frac{C}{K_C + C} - d_D D, \tag{4.2}$$

where δ_D is the diffusion coefficient, and d_D is the death rate of dendritic cells.

Naive CD4$^+$ T cells, Th0, can differentiate into several types of cells: Th_1, Th_2, Th_{17} and T regulatory cells (T_r). In the context of cancer the most relevant types are Th_1 (T_1) and T_r: T_1 is anti-cancer and T_r is pro-cancer.

Dentritic cells produce IL-12 and IL-12 activates T_1, while T_r resists the activation of T_1. Hence the differentiation of naive Th0 cells, T_0, into T_1 cells is proportional to

$$T_0 \frac{I_{12}}{K_{I_{12}} + I_{12}} \cdot \frac{1}{1 + T_r / K_{T_r T_1}},$$

where the inhibition of T_1 by T_r is represented by the factor $1/(1 + T_r/K_{T_r T_1})$.

T_1 cells are known to produce IL-2 which then attaches to these cells and promotes their replication. Thus, T_1 cells proliferate at a rate proportional to

$$T_1 \frac{I_2}{K_{I_2} + I_2};$$

here we used the Michaelis-Menten law to account for the receptor recycling time.

We can now write the equation for T_1 as follows:

$$\begin{aligned} \frac{\partial T_1}{\partial t} + \nabla \cdot (\vec{u} T_1) - \delta_{T_1} \nabla^2 T_1 =& \lambda_{T_1 I_{12}} T_0 \frac{I_{12}}{K_{I_{12}} + I_{12}} \frac{1}{1 + T_r / K_{T_r T_1}} \\ &+ \lambda_{T_1 I_2} T_1 \frac{I_2}{K_{I_2} + I_2} - d_{T_1} T_1, \end{aligned} \tag{4.3}$$

where δ_{T_1} is the diffusion coefficient of T_1 cells, and d_{T_1} is the death rate of T_1 cells.

For CD8$^+$ T cells we have a similar equation,

$$\begin{aligned} \frac{\partial T_8}{\partial t} + \nabla \cdot (\vec{u} T_8) - \delta_{T_8} \nabla^2 T_8 =& \lambda_{T_8 I_{12}} T_{80} \frac{I_{12}}{K_{I_{12}} + I_{12}} \frac{1}{1 + T_r / K_{T_r T_8}} \\ &+ \lambda_{T_8 I_2} T_8 \frac{I_2}{K_{I_2} + I_2} - d_{T_8} T_8. \end{aligned} \tag{4.4}$$

The reason why we do not combine T_1 and T_8 into one variable is that T_1 cells produce I_2 and do not kill cancer cells effectively, while T_8 cells do not produce I_2 but kill cancer cells effectively.

Cytokine TGF-β stimulates the production of Tregs; hence the equation for T_r has the following form:

$$\frac{\partial T_r}{\partial t} + \nabla \cdot (\vec{u} T_r) - \delta_{T_r} \nabla^2 T_r = \lambda_{T_r T_\beta} T_0 \frac{T_\beta}{K_{T_\beta} + T_\beta} - d_{T_r} T_r. \tag{4.5}$$

Since T_r inhibits the proliferation of T_1 and T_8 cells, it acts to promote cancer. And since T_β activates T_r, it is a pro-cancer cytokine and hence it is a target of anti-cancer drugs.

We assume that cancer cells undergo a logistic growth,

$$\text{constant} \times C \left(1 - \frac{C}{C_0}\right),$$

and they are killed by CD8$^+$ T cells at rate constant $\times T_8 C$; we neglect the killing of cancer cells by T_1. Cancer cells die, either by apoptosis or by necrosis, at a rate d_C. Hence,

$$\frac{\partial C}{\partial t} + \nabla \cdot (\vec{u}C) - \delta_C \nabla^2 C = \lambda_C C \left(1 - \frac{C}{C_0}\right) - d_{CT_8} T_8 C - d_C C. \tag{4.6}$$

We next turn to the dynamics of the cytokines. Since cytokines are much smaller than cells, their diffusion coefficients are several order of magnitude larger than those of cells. Hence the advection term $\nabla \cdot (\vec{u}X)$ for a cytokine X is negligible compared to its diffusion term $\delta_X \nabla^2 X$, and may therefore be dropped out.

It is known that I_{12} is produced by activated dendritic cells, I_2 is produced by T_1 cells, and T_β is produced by cancer cells and by T_r cells. Hence we have the following equations:

$$\frac{\partial I_{12}}{\partial t} - \delta_{I_{12}} \nabla^2 I_{12} = \lambda_{I_{12}D} D - d_{I_{12}} I_{12}, \tag{4.7}$$

$$\frac{\partial I_2}{\partial t} - \delta_{I_2} \nabla^2 I_2 = \lambda_{I_2 T_1} T_1 - d_{I_2} I_2, \tag{4.8}$$

$$\frac{\partial T_\beta}{\partial t} - \delta_{T_\beta} \nabla^2 T_\beta = \lambda_{T_\beta C} C + \lambda_{T_\beta T_r} T_r - d_{T_\beta} T_\beta. \tag{4.9}$$

Notice that the velocity $\vec{u}$ has not yet been determined and, at the same time, we have not yet exploited the assumption (4.1). In order to derive an equation for $\vec{u}$ we make two assumptions.

The first assumption is that all the cell types in Eq. (4.1) have the same diffusion coefficient, that is,

$$\delta_D = \delta_{T_1} = \delta_{T_8} = \delta_{T_r} = \delta_C.$$

If we then add Eqs. (4.2)-(4.6) and use Eq. (4.1), we get an equation for $\nabla \cdot \vec{u}$, namely,

$$\theta \nabla \cdot \vec{u} = \sum_{j=2}^{6} (\text{R.H.S. of Eq.}(4.j)).$$

The second assumption is about the tissue where the tumor is growing. We assume that it has the structure of a porous medium. This means that the velocity $\vec{u}$ is related to the pressure p among the cells by Darcy's law,

$$\vec{u} = -\nabla p.$$

Hence

$$\nabla^2 p = -\frac{1}{\theta} \sum_{j=2}^{6} (\text{R.H.S. of Eq.}(4.j)). \tag{4.10}$$

We proceed to impose boundary conditions on all the variables in Eqs. (4.2)-(4.10).

We assume that naive T cells from the lymph nodes migrate into the tumor and that their density, at the tumor boundary $\partial\Omega(t)$, is a constant, $\hat{T}_0$. Under the I_{12} environment these cells are induced to become T_1 cells, while under T_β environment

these cells are induced to become T_r cells. Hence we have the flux conditions:

$$\frac{\partial T_1}{\partial n} + \alpha \frac{I_{12}}{K_{I_{12}} + I_{12}}(T_1 - \hat{T}_0) = 0 \quad \text{on} \quad \partial\Omega(t), \tag{4.11}$$

$$\frac{\partial T_r}{\partial n} + \alpha \frac{T_\beta}{K_{T_\beta} + T_\beta}(T_r - \hat{T}_0) = 0 \quad \text{on} \quad \partial\Omega(t), \tag{4.12}$$

for some $\alpha > 0$, where $\partial/\partial n$ is the derivative in the direction of the outward normal. Similarly we take

$$\frac{\partial T_8}{\partial n} + \alpha \frac{I_{12}}{K_{I_{12}} + I_{12}}(T_8 - \hat{T}_{80}) = 0 \quad \text{on} \quad \partial\Omega(t), \tag{4.13}$$

for some constant density $\hat{T}_{80}$.

We next assume that D and C, as well as all the cytokines, satisfy a no-flux condition on the boundary of the tumor:

$$\frac{\partial X}{\partial n} = 0 \quad \text{for} \quad X = D, C, I_{12}, I_2, T_\beta \quad \text{on} \quad \partial\Omega(t). \tag{4.14}$$

It remains to prescribe a boundary condition for the pressure p. To do that we use two facts: (i) tumor tissue is more dense than the tissue surrounding it, and (ii) there are adhesive forces between cells. It follows that the cell-to-cell adhesion at the tumor boundary produces surface tension, and we express it in the form

$$p = \eta\kappa \quad \text{on } \partial\Omega(t) \quad (\eta > 0), \tag{4.15}$$

where η is proportional to the adhesive forces of the cells, and κ is the mean curvature; $\kappa = 1/R(t)$ if $\Omega(t)$ is a ball of radius $R(t)$.

If we denote by $\vec{n}$ the outward normal to the boundary $\partial\Omega(t)$, then the velocity of the cells at the boundary is $\vec{u} \cdot \vec{n}$. We assume that this is also the velocity of the boundary points. Hence

$$V_n = -\frac{\partial p}{\partial n} \quad \text{on } \partial\Omega(t) \tag{4.16}$$

where V_n is the velocity of the boundary points in the direction of the outward normal. Since the free boundary $\partial\Omega(t)$ is moving with velocity u, the advection terms do not appear in the flux conditions for cells.

We assume that the total density of cells is approximately 0.4 g/cm^3 (i.e. $\theta \sim$ 0.4 g/cm^3).

We finally prescribe initial conditions, noticing that the densities of D and the T cells is typically much smaller than the density of the cancer cells; for example:

$$\begin{aligned} &D = 1.2 \times 10^{-5}\text{g/cm}^3,\ T_1 = T_8 = 4 \times 10^{-3}\text{g/cm}^3, \\ &T_r = 1 \times 10^{-3}\text{g/cm}^3,\ C = 0.3956\text{g/cm}^3, \end{aligned} \tag{4.17}$$

and, correspondingly, take in Eqs. (4.1) and (4.10),

$$\theta = 0.404612\text{g/cm}^3. \tag{4.18}$$

We expect that the choice of the initial values does not appreciately affect the simulations of the model after a relatively short time.

So far our model does not include the TGF-β inhibitor, I. This drug inhibits the production of T_β in Eq. (4.9). We can express its effect by modifying Eq. (4.9)

as follows:

$$\frac{\partial T_\beta}{\partial t} - \delta_{T_\beta}\nabla^2 T_\beta = (\lambda_{T_\beta C}C + \lambda_{T_\beta T_r}T_r)\frac{1}{1+I/K_{T_\beta I}} - d_{T_\beta}T_\beta, \tag{4.19}$$

where $K_{T_\beta I}$ is a constant. The drug, taken in pills, circulates in the blood. It is depleted as it is absorbed by C and T_r cells, while some of it is washed out at rate d_I. If we represent by $\gamma(t)$ the source of the drug from the capillary system and by $\Gamma(t)$ the flux of the drug at the tumor boundary, then we have the following equation:

$$\frac{\partial I}{\partial t} - \delta_I\nabla^2 I = \gamma(t) - d_{ICT_r}\frac{I}{K_I + I} - d_I I \quad \text{in } \Omega(t), \tag{4.20}$$

where d_{ICT_r} is the rate of drug absorption by the C and T_r cells, and the boundary condition

$$\frac{\partial I}{\partial n} = \Gamma(t) \quad \text{on } \partial\Omega(t). \tag{4.21}$$

In order to simulate the model and determine the efficacy of the drug we need to first estimate all the parameters that appear in the system (4.1)-(4.21). This will be done in the next chapter.

Cancer model with GM-CSF

The cancer model (4.1)-(4.16) was developed in order to study the efficacy of TGF-β inhibitor as anti-cancer drug, We shall now proceed to describe a different mathematical model aimed at studying the effect of a different drug, namely, **granular macrophage colony stimulating factor** (GM-CSF).

Since cancer cells proliferate abnormally fast, they require more oxygen and other nutrients than the normal capillary system can provide. So cancer cells initiate a process, called **angiogenesis**, that leads to a new supply of blood. They secrete **vascular endothelial growth factor** (VEGF), a chemokine that attracts endothelial cells. Tips consisting of endothelial cells begin to grow and move toward the cancer and eventually form new blood vessels that provide additional oxygen and other nutrients to the tumor.

There are several anti-VEGF drugs that aim to block angiogenesis, and thus starve the cancer and suppress its growth. Here we focus on an experimental drug, GM-CSF. The drug was administrated to **nude mice**, that is, to mice whose immune system lacks T cells. We introduce a model which is a simplification of one developed in [**2**, **45**]. The model includes the following species:

C = cancer cells, E = endothelial cells, M = macrophages, V = VEGF, W = oxygen, P = MCP-1, F = M-CSF, G = GM-CSF, and soluble VEGF receptor (sVEGFR). Fig. 4.2 is a diagram showing the network of interactions among these species.

Cancer cells secrete VEGF, M-CSF, and MCP-1. MCP-1 recruits macrophages into the tumor microenvironment. The macrophages, like the tumor, also produce VEGF and MCP-1, both productions being facilitated by M-CSF. We note that the tumor has 'manipulated' cells of the immune system, namely, the macrophages, so that they increase the VEGF production. We refer to these tumor-manipulated macrophages as **tumor associated macrophages** (TAM), but we will not include

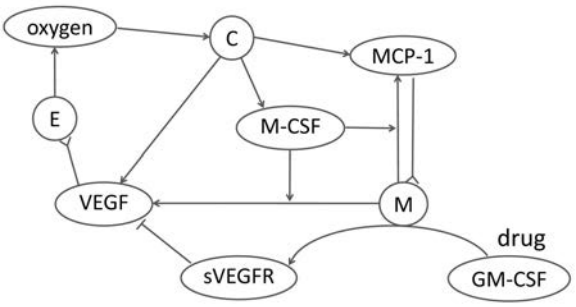

FIGURE 4.2. Network of cells and cytokines; sharp arrows represent reproduction/activation/enhancement, the blocked arrow represents blocking, and inverted sharp arrows represent chemoattraction.

in our model the actual process of how the tumor changes the phenotype of the macrophages to become TAM.

When the drug GM-CSF is absorbed by macrophages, it induces the macrophages to produce sVEGFR, which sequesters VEGF by binding to it. This neutralizes the pro-cancer activity of the tumor associated macrophages.

Setting R =sVEGFR, we can represent the dynamics associated with Fig. 4.2 by the following equations:

$$\begin{aligned}
&\frac{\partial C}{\partial t} + \nabla \cdot (\vec{u}C) - \delta_C \nabla^2 C = \lambda(W)C\left(1 - \frac{C}{C_0}\right) - d_C C,\\
&\frac{\partial E}{\partial t} + \nabla \cdot (\vec{u}E) - \delta_E \nabla^2 E = \lambda_E(V) - \nabla \cdot (E\nabla V) - d_E E,\\
&\frac{\partial M}{\partial t} + \nabla \cdot (\vec{u}M) - \delta_M \nabla^2 M = \lambda_M(C) - \nabla \cdot (M\nabla P) - d_M M,\\
&\frac{\partial P}{\partial t} - \delta_P \nabla^2 P = \lambda_{PC} C + \lambda_{PM} M\left(1 + \lambda_{PF}\frac{F}{K_F + F}\right) - d_P P,\\
&\frac{\partial W}{\partial t} - \delta_W \nabla^2 W = \lambda_{WE} E - \delta_{WC} C - \delta_{WM} M - d_W W,\\
&\frac{\partial F}{\partial t} - \delta_F \nabla^2 F = \lambda_{FC} C - \delta_{FM} M\frac{F}{K_F + F} - d_F F,\\
&\frac{\partial V}{\partial t} - \delta_V \nabla^2 V = \lambda_{VC} C + \lambda_{VM} M\left(1 + \lambda_{VF}\frac{F}{K_F + F}\right) - \delta_{VR} VR - d_V V,\\
&\frac{\partial R}{\partial t} - \delta_R \nabla^2 R = Mf(t) - \delta_{VR} VR - d_R R,
\end{aligned}$$

where $f(t)$ is the effect of the GM-CSF drug, injected into the tumor, on the production of sVEGFR by M. The equations for E and M include chemoattractant terms; the proliferation rate of C depends on oxygen concentration, $\lambda_C(W) = 0$ if W is below a critical value; the macrophages proliferation rate $\lambda_M(C)$ depends on C in somewhat complicated way; and the absorption of M-CSF (F) by M appears in the equations for P and V as enhancement terms and in the equation for M-CSF as a loss term. We note that the process of angiogenesis includes a proliferation of

endothelial cells, represented by the production rate $\lambda_E(V)$, which is a monotone increasing function of V.

The above model can be used to study the effect of the drug GM-CSF in nude mice. However, if we wish to study its effect on normal mice (i.e., on wild type mice) then we need to include T cells in the model.

Minimal models.

We develop a mathematical model in order to address a specific biological question. Since there is always uncertainty in estimating some of the model parameters (as will be seen in Chapter 5), the model should be "minimal". That is, the model should include all the biological species (variables) that are absolutely necessary in order to address the biological question, but exclude species that are thought to affect only little the answer to the biological question.

The decision what to include and what to exclude in order to build a minimal model is a judgement call, and we can illustrate it in the case of the model associated with Fig. 4.1. We recall that our focus in the model of Fig. 4.1 was on studying the effect of the drug TGF-β inhibitor. Since TGF-β activates T_r, and T_r secretes TGF-β, we had to include T_r in the model, and hence also the T cells which T_r inhibits, and, in particular, the T_8 cells which directly kill cancer cells. But macrophages do not directly kill cancer cells, although they are involved in changing the microenvironment of the tumor, as seen from Fig. 4.2. Since our aim was to compare the growth of cancer with or without an anti-TGF-β drug, it seemed reasonable to expect that the results on the efficacy of anti-TGF-β will not be significantly affected by the influence of macrophages on the tumor microenvironment. For this reason we excluded macrophages, and similarly also endothelial cells, VEGF and other species that appear in Fig. 4.2, when we developed the minimal model which focused on the efficacy of anti-TGF-β in reducing cancer growth.