Electronic Supplementary Material for "Investigating Nano-Sized Tumor-Derived Extracellular Vesicles in Enhancing Anti-PD-1 Immunotherapy"

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Tables and Equations

Table 1: Cellular species in the interaction network.

Cellular Species	Description	Mathematical representation
CD4+ Th0	Naive helper (CD4+) T-cell population	T_{N4}
CD4+ Th1	Type 1 helper T-cell population	Th_1
CD4+ Th2	Type 2 helper T-cell population	Th_2
CD4+ Treg	Regulatory T-cell population	T_{reg}
Naive CD8+	Naive cytotoxic (CD8+) T-cell population	T_{N8}
CD8+ Tc	Cytotoxic (CD8+) T-cell population	T_c
DC	Dendritic cell population	DC
NK	Natural killer cell population	NK
Cancer	Cancer cell population	C

Table 2: Cytokines, TDEV content, and drug species in the interaction network.

Protein Species	Description	Mathematical Representation
IL-4	Concentration of interleukin 4	[IL-4]
IL-6	Concentration of interleukin 6	[IL-6]
IL-12	Concentration of interleukin 12	[IL-12]
$IFN-\gamma$	Concentration of interferon gamma	$[IFN-\gamma]$
$TGF-\beta$	Concentration of tumor growth factor	$[TGF-\beta]$
PD-1	Concentration of programmed cell death protein 1	[PD-1]
PD-L1	Concentration of programmed death-ligand 1	[PD-L1]
Extracellular Vesicles Species	Description	Mathematical Representation
TDEV	Concentration of total extracellular vesicles	$[E_c]$
miRNA-21	Concentration of miRNA-21	[m21]
miRNA-203	Concentration of miRNA-203	[m203]
miRNA-214	Concentration of miRNA-214	[m214]
HSP-70	Concentration of heat shock protein 70	[HSP70]
Drug Species	Description	Mathematical Representation
Nivolumab	Concentration of the PD-1 inhibitor Nivolumab	[A]
Drug/PD-1 complex	Concentration of Nivolumab:PD-1 complex	[A: PD-1]

Table 3: Initial protein levels, and initial cells populations

Protein Species	Nominal Value	Range	Units	Reference
IL-4	11	0 - 61.37	pg/ml	Smalley et al.[1]
IL-6	3339.16	0 - 35884.0	pg/ml	Smalley et al.[1]
IL-12	1.54	0 - 11.44	pg/ml	Smalley et al.[1]
IFN-γ	0.45	0 - 482.31	pg/ml	Smalley et al.[1]
TGF-β	1000	0 - 4000	pg/ml	Geils et al.[2] - Hawinkels et al. -[3] Kohla et al.[4]
PD-1	9.9	-	(pg/ml)/cell	Smalley et al.[1]
PD-L1	1	-	(pg/ml)/cell	Smalley et al.[1]
Extracellular Species	Nominal Value	Range	Units	Reference
TDEV	3600	1000 - 4000	pg/ml	Friedman et al.[5]
miRNA-21	1.4	0.5 - 2	pg/ml	Friedman et al.[5]
miRNA-203	0.84	0.5 - 1.5	pg/ml	Friedman et al.[5]
miRNA-214	1.5	0.5 - 1.5	pg/ml	Estimated based on Friedman et al.[5] - Yin et al.[6]
HSP-70	1	1 - 4	ng/ml	Chanteloup et al.[7]
Cell Fractions	Nominal Value	Range	Units	Reference
T_{N4}	0.00292	0.001 - 0.69	-	Smalley et al.[1] - Friedman et al.[5]
Th_1	0.00486	0.001 - 0.99	-	Smalley et al.[1] - Friedman et al.[5]
Th_2	0.00973	0.001 - 0.99	-	Smalley et al.[1]
T_{reg}	0.00121	0.001 - 0.99	-	Friedman et al.[5]
T_{N8}	0.00194	0.001 - 0.97	-	Smalley et al.[1] - Friedman et al.[5]
T_c	0.00486	0.0 - 0.59	-	Smalley et al.[1] - Friedman et al.[5]
NK	0.00121	0.001 - 0.99	-	Bindea et al.[8] - Stankovic et al.[9]
DC	1e-5	0.001 - 0.99	-	Smalley et al.[1]
C	0.97322	0.1 - 0.97	-	Smalley et al.[1] - Friedman et al.[5]
Drug	Nominal Value	Range	Units	Reference
Nivolumab	240	N/A	mg q2Weeks	Samlowski et al.[10]

Naive CD4 T cells

The first term in the second equation shows the proliferation of naive CD4 T cells[1] (term #1). Naive CD4 T cells are differentiated into Th1, Th2, and T_{reg} T cells. TGF-β inhibits differentiation of Th1 T cells, and it is associated with reduced IL-12 receptor $\beta 2$.[11–13] PD-L1 inhibits differentiation of Th1 and Th2 T cells[14–16] (term #2 and #3). TGF-β helps differentiation of naive T cells into T_{reg}.[17,18] This differentiation is promoted by PD-L1 [TGF-β independent or in synergy with TGF-β];[19] however, in the presence of TGF-β, IL-6 blocks this differentiation into T_{reg}, and directs it into helper type 17 lineage[20] (term #4).

$$
\begin{aligned}
\frac{dT_{N_4}}{dt} &= \underbrace{n_4 T_{N4}}_{\text{growth of naive CD4 T cells}} \\
&- \underbrace{\left(d_{1-12} N4 \frac{[IL-12]}{q_{dIL12} + [IL-12]} \frac{r_{TGF}}{r_{TGF} + [TGF]} + d_{1-IFN} T_{N4} \frac{[IFN\gamma]}{q_{IFN} + [IFN\gamma]} \right)}_{\text{Th1 T cells differentiation}} \\
&\times \underbrace{\left(\frac{s_1}{s_1 + [PD-1 : PD-L1]} \right)}_{\text{Th1 T cells differentiation}} \\
&- \underbrace{\left(d_2 T_{N4} \frac{[IL-4]}{q_{dIL4} + [IL-4]} \right) \left(\frac{s_2}{s_2 + [PD-1 : PD-L1]} \right)}_{\text{Th2 T cells differentiation}} \\
&- \underbrace{\left(d_{T_{reg}-TGF} T_{N4} \frac{[TGF]}{q_{TGF} + [TGF]} + d_{T_{reg}-TGF} T_{N4} \frac{[TGF]}{q_{TGF} + [TGF]} \cdot \frac{[PD-L1]}{s_3 + [PD-L1]} \right)}_{\text{regulatory T cells differentiation (with synergistic effect of PD-L1 and without it)}} \\
&\times \underbrace{\left(\frac{r_{IL-6}}{r_{IL-6} + [IL-6]} \right)}_{\text{regulatory T cells differentiation (with synergistic effect of PD-L1 and without it)}} \\
&- \underbrace{\delta_{T_{N4}} [T_{N4}]}_{\text{natural death}} \qquad (1)
\end{aligned}
$$

Helper type 1 CD4 T cells

The first term in the second equation shows the proliferation of Th1 cells[1] (term #1). The second term describes the differentiation of the Th1 cell from naive CD4 T cells into Th1 cells in the presence of and IFN-γ[21] and IL-12,[22] which can be inhibited by TGF-β,[11–13] . Both of these differentiation promoters are inhibited by the PD-1:PD-L1 complex[14–16] (term #2).

$$\begin{aligned}
\frac{dTh_1}{dt} &= \underbrace{n_1 Th_1}_{\text{growth of Th1 T cells}} \\
&+ \underbrace{\left(d_{1-12}N4\frac{[IL-12]}{q_{dIL12}+[IL-12]}\frac{r_{TGF}}{r_{TGF}+[TGF]} + d_{1-IFN}T_{N4}\frac{[IFN\gamma]}{q_{IFN}+[IFN\gamma]}\right)}_{\text{Th1 T cells differentiation}} \\
&\times \underbrace{\left(\frac{s_1}{s_1+[PD-1:PD-L1]}\right)}_{\text{Th1 T cells differentiation}} \\
&- \underbrace{\delta_{Th_1}[Th_1]}_{\text{natural death}}
\end{aligned} \tag{2}$$

Helper type 2 CD4 T cells

The first term in the following equations is related to the differentiation of the Th2 cells from naive CD4 T cells into Th2 cells with the help of IL-4[21,22] (term #1). The second terms is added for the mitosis-dependent proliferation of Th2 cells which can be upregulated by IL-4[21,22] and inhibited by IFN-γ[23] and the PD-1:PD-L1 complex[14–16] (term #2).

$$\frac{dTh_2}{dt} = \underbrace{\left(d_2 T_{N4}\frac{[IL-4]}{q_{dIL4}+[IL-4]}\right)\left(\frac{s_2}{s_2+[PD-1:PD-L1]}\right)}_{\text{Th2 T cells differentiation}}$$

$$+\underbrace{\left(g_2 Th_2 + g_{2-4} Th_2 \frac{[IL-4]}{q_{gIL4}+[IL-4]}\right)\left(\frac{r_{IFN}}{r_{IFN}+[IFN\gamma]}\right)}_{\text{Th2 T cells Proliferation (IL-4 dependent and IL-4 independent)}} - \underbrace{\delta_{Th_2}[Th_2]}_{\text{natural death}} \qquad (3)$$

Regulatory CD4 T cells

The first term corresponds for the proliferation of $\mathrm{T_{reg}}$ cells (term #1). TDEV miRNA-214 helps the expansion of CD4+CD25+Foxp3+ $\mathrm{T_{reg}}$ in the TME by downregulation of the PTEN-mediated signalling cascade. PTEN is a negative modulator of $\mathrm{T_{reg}}$ homeostasis *in vivo* and expansion *ex vivo*[6] (term #2). TGF-β is necessary for $\mathrm{T_{reg}}$ initial differentiation from naive CD4+ T cells[17,18] (term #3).

$$\frac{dT_{reg}}{dt} = \underbrace{n_{reg}T_{reg}}_{\text{growth of regulatory T cells}} + \underbrace{\left(n_{reg-214}T_{reg}\frac{[miR214]}{q_{214}+[miR214]}\right)}_{\text{expansion by miRNA-214}}$$

$$+\underbrace{\left(d_{T_{reg}-TGF}T_{N4}\frac{[TGF]}{q_{TGF}+[TGF]} + d_{T_{reg}-TGF}T_{N4}\frac{[TGF]}{q_{TGF}+[TGF]}\frac{[PD-L1l]}{s_3+[PD-L1]}\right)}_{\text{regulatory T cells differentiation (with synergistic effect of PD-L1 and without it)}}$$

$$\times \underbrace{\left(\frac{r_{IL-6}}{r_{IL-6}+[IL-6]}\right)}_{\text{regulatory T cells differentiation (with synergistic effect of PD-L1 and without it)}} - \underbrace{\delta_{T_{reg}}[T_{reg}]}_{\text{natural death}} \qquad (4)$$

Naive CD8 T cells

In the following equation the first term corresponds for the proliferation of naive CD8 T cells[1] (term #1). The next term describes the product of naive CD8 differentiation into cytotoxic CD8 T cells with the help of Th1 T cells,[24,25] which can be inhibited by PD-L1 binding to the PD-1[14–16] (term #2).

$$\frac{dT_{N8}}{dt} = \underbrace{n_8 T_{N8}}_{\text{growth of naive CD8 T cells}} \underbrace{- d_c T_{N8}(\frac{Th_1}{q_1 + Th_1})(\frac{s_c}{s_c + [PD-1:PD-L1]})}_{\text{differentiation of CD8 T cells}}$$

$$- \underbrace{\delta_{T_{N8}}[T_{N8}]}_{\text{natural death}} \tag{5}$$

Cytotoxic CD8 T cells

In the equation for the cytotoxic CD8 T cells, the first term describes the proliferation of cytotoxic T cells, which can be positively influenced by IL-12[26] (terms #1 and #2). The final term represents the cytotoxic CD8 T cells being differentiated from the naive CD8 T cells with help of Th1 cells, which can be inhibited by PD-1:PD-L1 complex[14–16] (term #3).

$$\frac{dT_c}{dt} = \underbrace{n_c T_c}_{\text{growth of cytotoxic CD8 T cells}} \underbrace{+ g_{c-12} T_c(\frac{[IL-12]}{q_{gIL12} + [IL-12]})}_{\text{growth of cytotoxic CD8 T cells}}$$

$$\underbrace{+ d_c T_{N8}(\frac{Th_1}{q_1 + Th_1})(\frac{s_c}{s_c + [PD-1:PD-L1]})}_{\text{differentiation of CD8 T cells}} - \underbrace{\delta_{T_c}[T_c]}_{\text{natural death}} \tag{6}$$

Natural killer cells

In the adaptive immune responses, NK cells are activated by Th1-type cytokines such as IL-2, -12, or -18[27] (term #1).

$$\frac{d[NK]}{dt} = \underbrace{g_{NK-12}NK\left(\frac{[IL-12]}{q_{gIL12}+[IL-12]}\right)}_{\text{IL-12-dependent proliferation of natural killer cells}} - \underbrace{\delta_{NK}\,[NK]}_{\text{natural death}} \tag{7}$$

Dendritic cells

DCs' proliferation can be effected by necrotic cancer cells lysates such as release danger associated molecular patterns (DAMPs) including heat shock proteins and high mobility group box protein 1 (HMGB1).[28,29] Moreover, results demonstrate that TDEV miRNA-203 can adversely regulate the Toll-like Receptor 4 in dendritic cells[30] which can affect their maturation[31] (term #1). Also, Th1 T cells help activation of dendritic cells[24] (term #2).

$$\frac{d[DC]}{dt} = \underbrace{g_{DC-cancer}C\left(\frac{r_{203}}{r_{203}+[miR203]}\right)}_{\text{maturation by cancer cells}} + \underbrace{g_{DC-Th_1}\,[Th1]}_{\text{maturation by Th1 cells}} - \underbrace{\delta_{DC}\,[DC]}_{\text{natural death}} \tag{8}$$

Cancer cells

In the equation for cancer cells, the first term corresponds to their natural growth (term #1).[1] TDEV miRNA-21 has been shown to have a pro-tumor effect through different mechanisms[32–34] (term #2). Cytotoxicity of NK cells[35] and cytotoxic $CD8^+$ cells[36] toward cancer cells is mediated by many different mechanisms, among which perforin/granzyme cytotoxicity is the most effective way (term #3 and #4).

$$\frac{dC}{dt} = \underbrace{n_{cancer}C}_{\text{growth of cancer cells}} + \underbrace{n_{cancer-21}C\frac{[miR21]}{q_{21}+[miR21]}}_{\text{expansion by miR-21}} - \underbrace{k_c C T_c}_{\text{elimination by cytotoxic T cells}}$$

$$- \underbrace{k_{NK}C.NK}_{\text{elimination by natural killer cells}} - \underbrace{\delta_C\,[C]}_{\text{natural death}} \tag{9}$$

Equations for Cytokines

TGF-β

The TGF-β present in this model has been supposed to be produced mainly by T_{reg}, which is in agreement with the literature as it has been shown to protect the role of T_{reg} in the TME as immune tolerance regulator and promotes its homeostasis.[37–41]

$$\frac{d[TGF-\beta]}{dt} = \underbrace{p_{Treg-TGF}Treg}_{\text{production by }T_{reg}\text{ cells}} - \underbrace{\delta_{TGF}\,[TGF]}_{\text{natural decay}} \tag{10}$$

IFN-γ

IFN-γ is produced by most of the anti-tumor immune cells here in this model. The first term shows the production of IFN-γ by Th1 T cells[21–23] which is inhibited by IL-4[42] and IL-6[21] and T_{reg}.[43] T_{reg} suppresses IFN-γ expression of Th1 cells[43,44] (term #1). TGF-β hampers production of IFN-γ by CD8$^+$[45] and NK cells [*in vivo*,[46,47] and IL-12 dependent[48–50] productions (term #2 and #3). TDEV HSP70 enhances the secretion of IFN-γ by NKs[51,52] (term #3).

$$\frac{d\,[IFN-\gamma]}{dt} = \underbrace{p_{1-IFN}Th_1\left(\frac{r_{IL4}}{r_{IL4}+[IL4]}\right)\left(\frac{r_{IL6}}{r_{IL6}+[IL-6]}\right)\left(\frac{r_{Treg}}{r_{Treg}+[Treg]}\right)}_{\text{production by Th1}}$$

$$+\underbrace{p_{c-IFN}T_c\left(\frac{r_{TGF}}{r_{TGF}+[TGF]}\right)}_{\text{production by CD8+ Tc}}$$

$$+\underbrace{p_{NK-IFN}NK\left(\frac{r_{TGF}}{r_{TGF}+[TGF]}+\frac{[HSP70]}{q_{HSP70}+[HSP70]}\right)}_{\text{production by NKs}}-\underbrace{\delta_{IFN}\,[IFN]}_{\text{decay rate}} \quad (11)$$

Interleukin 4

In this equation, the first term is for the production of IL-4 by Th2 cells[1] which can be inhibited by Treg cells[53,54] (term #1). The second term describes the production of IL-4 by Th2 cells in the presence of IL-6[1] (term #2).

$$\frac{d\,[IL-4]}{dt} = \underbrace{p_{2-4}Th_2\left(\frac{r_{Treg}}{r_{Treg}+[Treg]}\right)}_{\text{production by Th2}}+\underbrace{p_{2-4-6}Th_2\left(\frac{[IL-6]}{q_{IL6}+[IL-6]}\right)}_{\text{production by Th2 in the presence of IL-6}}$$

$$-\underbrace{\delta_{IL-4}\,[IL-4]}_{\text{decay rate}} \quad (12)$$

Interleukin 6

In this equation, the first term is for the production of IL-6 by Th2 cells[1](term #1). The second term describes the production of IL-6 by DC cells[1] (term #2).

$$\frac{d\left[IL-6\right]}{dt} = \underbrace{p_{2-6}Th_2}_{\text{production by Th2}} + \underbrace{p_{DC-6}DC}_{\text{production by antigen presenting cells}} - \underbrace{\delta_{IL-6}\left[IL-6\right]}_{\text{natural decay}} \tag{13}$$

Interleukin 12

In this equation, the first term is for the production of IL-12 by DC cells which TDEV miRNA-203 disrupts this cytokine's productions by DC cells[30](term #1). The second term describes the production of IL-12 by Th1 cells[1] (term #2). The third term describes the production of IL-12 by NK cells[1] (term #3).

$$\begin{aligned} \frac{d\left[IL-12\right]}{dt} &= \underbrace{p_{DC-12}DC\left(\frac{r_{203}}{r_{203}+[miR203]}\right)}_{\text{production by DCs}} + \underbrace{p_{1-12}Th_1}_{\text{production by Th1 cells}} \\ &+ \underbrace{p_{NK-12}NK}_{\text{production by NK cells}} - \underbrace{\delta_{IL12}\left[IL-12\right]}_{\text{natural decay}} \end{aligned} \tag{14}$$

Equations for TDEVs

Released TDEVs

TDEVs are mainly released from cancer cells. They contain HSP70,[52] PD-L1[55,56] miRNA-21,[57] miRNA-214,[6] and miRNA-203.[30] TDEVs miRNAs 21, 214, and 203 affect cancer cells, T_{reg}, and DCs respectively. miRNAs are released upon their contact with their target cells. TDEV miRNA-21, miRNA-203, and miRNA-214 are released when they are in contact with cancer cells, dendritic cells, and regulatory T cells, respectively. TDEV miRNA-21 has been shown to have anti-apoptotic effect in glioblastoma cells.[58] TDEV HSP70 enhances the secretion of IFN-γ by NKs.[51,52]

$$\frac{dE_c}{dt} = \underbrace{\lambda_{E_c} C}_{\text{production by cancer cells}} - \underbrace{\delta_{E_c} E_C}_{\text{natural decay}}$$

$$- \underbrace{d_{deg} E_c \left(\frac{[Treg]}{k_{T_{reg}} + [Treg]} + \frac{[DC]}{k_{DC} + [DC]} + \frac{[C]}{k_{can} + [C]} \right)}_{\text{degradation in contact with target cells}} \quad (15)$$

HSP70

$$\frac{d\,[HSP70]}{dt} = \underbrace{\lambda_{exoHSP70} E_c}_{\text{TDEV HSP70}} - \underbrace{\delta_{HSP70}\,[HSP70]}_{\text{natural decay}} \quad (16)$$

miRNA-21

$$\frac{d[m21]}{dt} = \underbrace{\lambda_{exo-m21} E_c \left(\frac{[C]}{k_{can} + [C]} \right)}_{\text{released in contact with cancer cells}} - \underbrace{\delta_{m21}\,[m21]}_{\text{natural decay}} \quad (17)$$

miRNA-214

$$\frac{d[m214]}{dt} = \underbrace{\lambda_{exo-m214} E_c \left(\frac{[Treg]}{k_{Treg} + [Treg]} \right)}_{\text{released in contact with regulatory T cells}} - \underbrace{\delta_{m214}\,[m214]}_{\text{natural decay}} \quad (18)$$

miRNA-203

$$\frac{d[m203]}{dt} = \underbrace{\lambda_{exo-m203} E_c \left(\frac{[DC]}{k_{DC} + [DC]}\right)}_{\text{released in contact with dendritic cells}} - \underbrace{\delta_{m203}\,[m203]}_{\text{natural decay}} \tag{19}$$

Equations for PD-1 and PD-L1

PD1

In this model, it has been assumed that all immune cells are expressing PD-1 in the same amount. The first term in the evolution of PD-1 shows this expression[1] (term #1). The second term shows its consumption to form the PD-1:PD-L1 complex[1] (term #2); this complex can be dissociated into PD-1 and PD-L1[1] (term #3). Finally, it can bind to the anti-PD-1 treatment drug to form the drug-PD-1 complex which can also be dissociated into its forming components (term #4).

$$\begin{aligned}\frac{d\,[PD-1]}{dt} &= \underbrace{\rho\left(\frac{dTh_1}{dt} + \frac{dTh_2}{dt} + \frac{dT_c}{dt} + \frac{dDC}{dt} + \frac{NK}{dt}\right)}_{\text{expression of PD-1 on Th1, Th2, Tc, DC and NK cells}} \\ &\quad - \underbrace{\beta_+\,[PD-1]\,[PD-L1]}_{\text{formation of the PD-1:PD-L1 complex}} + \underbrace{\beta_-\,[PD-1:PD-L1]}_{\text{dissociation of the PD-1:PD-L1 complex}} \\ &\quad - \underbrace{\alpha_+\,[PD-1]\,[A]}_{\text{binding of PD-1 to Nivolumab}} + \underbrace{\alpha_-\,[PD-1:A]}_{\text{dissociation of the Nivolumab:PD-1 complex}}\end{aligned} \tag{20}$$

PD-L1

In the modeling study by Friedman et al[5] it has been noted that the concentration of PD-L1 is unknown. So, in that work the concentration of TDEVs containing PD-L1, has been suggested to correlate with overall sEV concentration from cancer cells. However, in this model, to take the TDEV PD-L1 into account more accurately, an additional term has been added. $\lambda_{exo-PDL1}$ is the PD-L1 TDEV expression level adopted from the study by Chen et al.[55]

$$\frac{d\left[PD-L1\right]}{dt} = \underbrace{\lambda\left(\frac{dTh_1}{dt}+\frac{dTh_2}{dt}+\frac{dT_c}{dt}+\frac{dC}{dt}\right)}_{\text{expression of PD-L1 on Th1, Th2, T8, and cancer cells}} + \underbrace{\lambda_{cancer-IFN}\frac{dC}{dt}\left(\frac{[IFN]}{q_{IFN-PDL1}+[IFN]}\right)}_{\text{upregulation of PD-L1 in cancer by IFN-}\gamma}$$

$$+\underbrace{\lambda_{exo-PDL1}\frac{dE_c}{dt}}_{\text{TDEV PD-L1}} - \underbrace{\beta_+\left[PD-1\right]\left[PD-L1\right]}_{\text{formation of the PD-1:PD-L1 complex}} + \underbrace{\beta_-\left[PD-1:PD-L1\right]}_{\text{dissociation of the PD-1:PD-L1 complex}} \quad (21)$$

Equation for PD-1:PD-L1 Complex

Here, the first term describes the binding of the receptor PD-1 to its ligand, PD-L1 resulting in the PD-1:PD-L1 complex (term #1). The second term results from the dissociation of the PD-1:PD-L1 complex[16] (term #2).

$$\frac{d\left[PD-1:PD-L1\right]}{dt} = \underbrace{\beta_+\left[PD-1\right]\left[PD-L1\right]}_{\text{binding of PD-1 to PD-L1}} - \underbrace{\beta_-\left[PD-1:PD-L1\right]}_{\text{dissociation of PD-1:PD-L1 complex}} \quad (22)$$

Equation for Drug

In this equation, the first term describes the administration of the anti-PD-1 drug[10] (term #1). This drug can bind to the PD-1 receptor preventing its binding to PD-L1[1] (term #2).

The third term shows the dissociation of the drug-PD-1 complex[1] (term #3). The final term is for the natural decay of the drug in the system[1] (term #4). According to the literature,[10] 240 mg of Nivolumab is administered every two weeks for the duration of anti-PD1 therapy. Here, as a simplification, this quantity is divided into 14 equal portions (14 days), and it is being used by cells in an equal concentration during every day of the simulations.

$$\frac{dA}{dt} = \underbrace{\widetilde{A}(t)}_{\text{introduction of the drug into the TME}} - \underbrace{\alpha_+ [A] [PD-1]}_{\text{the binding of drug to PD-1}} + \underbrace{\alpha_- [A:PD-1]}_{\text{dissociation of the drug:PD-1 complex}} - \underbrace{\delta_A [A]}_{\text{natural decay}} \quad (23)$$

Equation for Drug:PD-1 Complex

In this equation, the first term is for the binding of the anti-PD1 drug, Nivolumab, to the PD-1 receptor forming the Nivolumab:PD-1 complex (term #1). The second term originates from the dissociation of the drug complex[15] (term #2).

$$\frac{d\,[A:PD-1]}{dt} = \underbrace{\alpha_+ [A] [PD-1]}_{\text{binding of drug with PD-1}} - \underbrace{\alpha_- [A:PD-1]}_{\text{dissociation of the drug complex}} \quad (24)$$

Table 4: Description of the kinetic parameters and their mathematical representation used in the model.

Parameter Number	Description	Mathematical Representation
1	proliferation rate of naïve CD4 cells	n_4
2	proliferation rate of naïve CD8 cells	n_8
3	proliferation rate of Th1 cells	n_1
4	proliferation rate of Treg cells	n_{reg}
5	miRNA214-dependent proliferation rate of Treg cells	$n_{reg-214}$
6	IL 12-independent proliferation rate of Tc cells	n_c
7	proliferation rate of cancer cells	n_{cancer}
8	miRNA21-dependent proliferation rate of cancer cells	$n_{cancer-21}$
9	IL 4-independent growth rate of Th2 cells	g_2
10	IL 4-dependent growth rate of Th2 cells	g_{2-4}
11	IL 12-dependent growth rate of Tc cells	g_{c-12}
12	IL 12-dependent growth rate of natural killer cells	g_{NK-12}
13	cancer cells-dependent growth rate of dendritic cells	$g_{DC-cancer}$
14	Th1 cells-dependent growth rate of dendritic cells	g_{DC-Th1}
15	IL 12-dependent differentiation rate of naïve CD4 cells into Th1 cells	d_{1-12}
16	IFN-γ-dependent differentiation rate of naïve CD4 cells into Th1 cells	d_{1-IFN}
17	IL 4-dependent differentiation rate of naïve CD4 cells into Th2 cells	d_2
18	TGF-β-dependent differentiation rate of naïve CD4 cells into Treg cells	$d_{Treg-TGF}$

Table 4 continued from previous page

Parameter Number	Description	Mathematical Representation
19	Differentiation rate of naïve CD8 cells into cytotoxic CD8 cells	d_c
20	Rate of cancer cell killing by Tc cells	k_c
21	Rate of cancer cell killing by natural killer cells	k_{NK}
22	Half-maximal PD-1:PD-L1 concentration for inhibition of naïve CD4 cells differentiation into Th1 cells	s_1
23	Half-maximal PD-1:PD-L1 concentration for inhibition of naïve CD4 cells differentiation into Th2 cells	s_2
24	Half-maximal PD-1:PD-L1 concentration for promotion of naïve CD4 cells differentiation into Treg cells	s_3
25	Half-maximal PD-1:PD-L1 concentration for inhibition of naïve CD8 cells differentiation into Tc cells	s_c
26	Half-maximal IL-12 concentration for IL12-dependent differentiation of naïve CD4 cells into Th1 cells	q_{dIL12}
27	Half-maximal IL-12 concentration for IL12-dependent proliferation of Tc and NK cells	q_{gIL12}
28	Half-maximal IFNγ concentration for IFNγ-dependent differentiation of naïve CD4 cells into Th1 cells	q_{IFN}
29	Half-maximal IL-4 concentration for IL4-dependent differentiation of naïve CD4 cells into Th2 cells	q_{dIL4}
30	Half-maximal IL-4 concentration for IL4-dependent proliferation of Th2 cells	q_{gIL4}
31	Half-maximal TGF-β concentration for TGF-β-dependent differentiation of Treg cells	q_{TGF}

Table 4 continued from previous page

Parameter Number	Description	Mathematical Representation
32	Half-maximal miR214 concentration for TDEV-dependent expansion of Tregs	q_{214}
33	Half-maximal HSP70 concentration for HSP70-dependent proliferation	q_{HSP70}
34	Half-maximal miR21 concentration for miR21-dependent expansion of cancer cells	q_{21}
35	Half-maximal Th1 cell population for naïve CD8 differentiation into Tc cells	q_1
36	Half-maximal IL-6 concentration for IL6-dependent production of IL-4 by Th2 cells	q_{IL6}
37	Half-maximal IFNγ concentration for IFNγ-dependent PD-L1 expression by cancer cells	$q_{IFN-PDL1}$
38	Half-maximal Treg population for Treg-dependent inhibition of IFN-γ production by Th1 cells and IL-4 production by Th2 cells	r_{Treg}
39	Half-maximal IFN-γ concentration for IFN-γ-dependent inhibition of Th2 proliferation	r_{IFN}
40	Half-maximal IL-4 concentration for IL4-dependent inhibition of IFNγ production by Th1 cells	r_{IL4}
41	Half-maximal IL-6 concentration for IL6-dependent inhibition of IFNγ production by Th1 cells	r_{IL6}
42	Half-maximal TGF-β concentration for inhibition of Th1 cells differentiation from naïve CD4 cells	r_{TGF}

Table 4 continued from previous page

Parameter Number	Description	Mathematical Representation
43	Half-maximal miR203 concentration for inhibition of cancer cell-dependent maturation of dendritic cells	r_{203}
44	Rate of TGF-β production by Treg cells	$p_{Treg-TGF}$
45	Rate of IFN-γ production by Th1 cells	p_{1-IFN}
46	Rate of IFN-γ production by Tc cells	p_{c-IFN}
47	Rate of IFN-γ production by NK cells	p_{NK-IFN}
48	Rate of IL6-independent production of IL-4 by Th2 cells	p_{2-4}
49	Rate of IL6-dependent production of IL-4 by Th2 cells	p_{2-4-6}
50	Rate of IL-6 production by Th2 cells	p_{2-6}
51	Rate of IL-6 production by dendritic cells	p_{DC-6}
52	Rate of IL-12 production by Th1 cells	p_{1-12}
53	Rate of IL-12 production by dendritic cells	p_{DC-12}
54	Rate of IL-12 production by Natural Killer cells	p_{NK-12}
55	Decay rate of TGF-β	δ_{TGF}
56	Decay rate of IFN-γ	δ_{IFN}
57	Decay rate of IL-6	δ_{IL6}
58	Decay rate of IL-4	δ_{IL4}
59	Decay rate of IL-12	δ_{IL12}
60	Decay rate of Nivolumab	δ_A
61	Decay rate of TDEVs	δ_{E_c}
62	Decay rate of miRNA21	δ_{m21}
63	Decay rate of miRNA214	δ_{m214}
64	Decay rate of miRNA203	δ_{m203}

Table 4 continued from previous page

Parameter Number	Description	Mathematical Representation
65	Decay rate of HSP70	δ_{HSP70}
66	Death rate of naïve CD4 cells	$\delta_{T_{N4}}$
67	Death rate of naïve CD8 cells	$\delta_{T_{N8}}$
68	Death rate of Th1 cells	δ_{Th_1}
69	Death rate of Th2 cells	δ_{Th_2}
70	Death rate of Treg cells	$\delta_{T_{reg}}$
71	Death rate of Tc CD8 cells	δ_{T_c}
72	Death rate of NK cells	δ_{NK}
73	Death rate of DC cells	δ_{DC}
74	Death rate of cancer cells	δ_C
75	Per-cell expression level of PD-1	ρ
76	Per-cell expression level of PD-L1	λ
77	IFN-γ-dependent PD-L1 expression per cancer cell	$\lambda_{can-IFN}$
78	Production rate of TDEV miRNA21	$\lambda_{exo-m21}$
79	Production rate of TDEV miRNA214	$\lambda_{exo-m214}$
80	Production rate of TDEV miRNA203	$\lambda_{exo-m203}$
81	Production rate of TDEV PD-L1	$\lambda_{exo-PD-L1}$
82	Production rate of TDEV HSP70	$\lambda_{exo-HSP70}$
83	Rate of association of PD-1 and PD-L1	$\beta+$
84	Rate of dissociation of PD-1:PD-L1 complex	$\beta-$
85	Rate of association of Nivolumab:PD-1 complex	$\alpha+$
86	Rate of dissociation of Nivolumab:PD-1 complex	$\alpha-$
87	Cancer cells saturation	k_{can}

Table 4 continued from previous page

Parameter Number	Description	Mathematical Representation
88	DC saturation	k_{DC}
89	Treg saturation	$k_{T_{reg}}$
90	Degradation rate of TDEVs by cells	d_{deg}

Table 5: Nominal values of the kinetic parameters implemented in the model.

Parameter Number	Nominal Value	Units	References
1	2.5×10^{-1}	day^{-1}	Smalley et al.[1]
2	$3.5 \times\ 10^{-1}$	day^{-1}	Smalley et al.[1]
3	$4.8 \times\ 10^{-2}$	day^{-1}	Smalley et al.[1]
4	$4.8 \times\ 10^{-2}$	day^{-1}	Estimated from Smalley et al.[1]
5	$1.0 \times\ 10^{-4}$	day^{-1}	Yin et al.[6]
6	$4.1 \times\ 10^{-2}$	day^{-1}	Smalley et al.[1]
7	$7.0 \times\ 10^{-2}$	day^{-1}	Smalley et al.[1]
8	6.4×10^{-1}	day^{-1}	Estimated
9	3.8×10^{-2}	day^{-1}	Smalley et al.[1]
10	3.5×10^{-2}	day^{-1}	Smalley et al.[1]
11	3.6×10^{-2}	day^{-1}	Estimated from Friedman et al.[5]
12	2.0×10^{-2}	day^{-1}	Estimated from Friedman et al.[5]
13	8.0×10^{-7}	day^{-1}	Friedman et al.[5]
14	8.0×10^{-7}	day^{-1}	Estimated from Friedman et al.[5]
15	3.6×10^{-2}	day^{-1}	Smalley et al.[1]
16	1.9×10^{-1}	day^{-1}	Smalley et al.[1]
17	2.1×10^{-2}	day^{-1}	Smalley et al.[1]
18	2.5×10^{-4}	day^{-1}	Zheng et al.[59]
19	2.3×10^{-2}	day^{-1}	Smalley et al.[1]
20	1.1×10^{-5}	$Tc \quad cell^{-1}.day^{-1}$	Smalley et al.[1]
21	1.1×10^{-5}	$NK \quad cell^{-1}.day^{-1}$	Estimated from Smalley et al.[1]
22	4.9×10^{1}	pg/ml	Smalley et al.[1]
23	2.1	pg/ml	Smalley et al.[1]

Table 5 continued from previous page

Parameter Number	Nominal Value	Units	References
24	2.0	pg/ml	Estimated from Smalley et al.[1]
25	1.9×10^1	pg/ml	Smalley et al.[1]
26	6.3×10^{-3}	pg/ml	Smalley et al.[1]
27	3.4×10^{-2}	pg/ml	Smalley et al.[1]
28	4.0×10^{-1}	pg/ml	Smalley et al.[1]
29	8.4×10^{-1}	pg/ml	Smalley et al.[1]
30	4.03	pg/ml	Smalley et al.[1]
31	4.0	pg/ml	Estimated from Friedman et al.[5]
32	2.0	pg/ml	Estimated from Friedman et al.[5]
33	2.0	pg/ml	Estimated from Friedman et al.[5]
34	2.0	pg/ml	Friedman et al.[5]
35	1.6×10^2	$Th1\ cells$	Smalley et al.[1]
36	1.3×10^2	pg/ml	Smalley et al.[1]
37	6.3×10^{-1}	pg/ml	Smalley et al.[1]
38	2.3×10^3	$Treg\ cells$	Estimated from Pace et al.[54]
39	8.9×10^{-2}	pg/ml	Smalley et al.[1]
40	7.5×10^{-1}	pg/ml	Smalley et al.[1]
41	1.4×10^2	pg/ml	Smalley et al.[1]
42	1.0×10^3	pg/ml	Estimated from Takimoto et al.[60]
43	1.8	pg/ml	Estimated from Friedman et al.[5]
44	1.25×10^{-3}	$\frac{pg/ml}{Treg\ cells.day}$	Estimated from Takimoto et al.[60]
45	1.3×10^{-3}	$\frac{pg/ml}{Th1\ cells.day}$	Smalley et al.[1]
46	6.2×10^{-4}	$\frac{pg/ml}{Tc\ cells.day}$	Estimated

Table 5 continued from previous page

Parameter Number	Nominal Value	Units	References
47	1.3×10^{-3}	$\frac{pg/ml}{NK\ cells.day}$	Estimated
48	3.7×10^{-6}	$\frac{pg/ml}{Th2\ cells.day}$	Smalley et al.[1]
49	1.6×10^{-4}	$\frac{pg/ml}{Th2\ cells.day}$	Smalley et al.[1]
50	1.1×10^{-1}	$\frac{pg/ml}{Tc\ cells.day}$	Smalley et al.[1]
51	2.2×10^{-2}	$\frac{pg/ml}{DC\ cells.day}$	Dodge et al.[61]
52	7.7×10^{-5}	$\frac{pg/ml}{Th1\ cells.day}$	Smalley et al.[1]
53	1.2×10^{-3}	$\frac{pg/ml}{DC\ cells.day}$	Smalley et al.[1]
54	1.2×10^{-3}	$\frac{pg/ml}{NK\ cells.day}$	Estimated
55	3.5×10^{-4}	min^{-1}	Menon et al.[62] - Kim et al.[63]
56	ln(2)/1000	min^{-1}	Smalley et al.[1]
57	ln(2)/1000	min^{-1}	Smalley et al.[1]
58	7.0×10^{-4}	min^{-1}	Smalley et al.[1]
59	4.8×10^{-4}	min^{-1}	Smalley et al.[1]
60	4.8×10^{-2}	min^{-1}	Smalley et al.[1]
61	1.0×10^{4}	min^{-1}	Estimated
62	1.5	day^{-1}	Friedman et al.[5]
63	1.5	day^{-1}	Estimated from Friedman et al.[5]
64	1.5	day^{-1}	Friedman et al.[5]
65	1.5	day^{-1}	Estimated from Friedman et al.[5]
66	1.0×10^{-2}	day^{-1}	Estimated from Smalley et al.[1]
67	1.0×10^{-2}	day^{-1}	Estimated from Smalley et al.[1]
68	1.2×10^{-2}	day^{-1}	Estimated from Smalley et al.[1]
69	1.2×10^{-2}	day^{-1}	Smalley et al.[1]

Table 5 continued from previous page

Parameter Number	Nominal Value	Units	References
70	1.2×10^{-2}	day^{-1}	Estimated from Smalley et al.[1]
71	1.2×10^{-2}	day^{-1}	Estimated from Smalley et al.[1]
72	1.2×10^{-2}	day^{-1}	Estimated from Smalley et al.[1]
73	1.2×10^{-2}	day^{-1}	Estimated from Smalley et al.[1]
74	1.7×10^{-1}	day^{-1}	Friedman et al.[5]
75	9.9	$\frac{pg/ml}{T\ cells}$	Smalley et al.[1]
76	1.0×10^{1}	$\frac{pg/ml}{cells}$	Smalley et al.[1]
77	1.8×10^{-4}	$\frac{pg/ml}{cancer\ cells}$	Smalley et al.[1]
78	3.8	day^{-1}	Estimated from Friedman et al.[5]
79	1	day^{-1}	Estimated from Friedman et al.[5]
80	1.32	day^{-1}	Friedman et al.[5]
81	50	day^{-1}	Chen et al.[55]
82	1	day^{-1}	Estimated from Friedman et al.[5]
83	8.43×10^{-4}	$((pg/ml)\,.day)^{-1}$	Smalley et al.[1]
84	700	day^{-1}	Smalley et al.[1]
85	1.4×10^{-1}	$((pg/ml)\,.day)^{-1}$	Smalley et al.[1]
86	7.0×10^{-4}	$((pg/ml)\,.day)^{-1}$	Smalley et al.[1]
87	4.5×10^{8}	$Cancer\ cells$	Friedman et al.[5]
88	23	$DC\ cells$	Friedman et al.[5]
89	1.6×10^{3}	$Treg\ cells$	Friedman et al.[5]
90	21.8	day^{-1}	Friedman et al.[5]

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