Supplementary Information

Normalizing tumor microenvironment with nanomedicine and metronomic therapy to improve immunotherapy

Fotios Mpekris, Chrysovalantis Voutouri, Myrofora Panagi, James W. Baish, Rakesh K. Jain and Triantafyllos Stylianopoulos

Cell culture. 4T1 (ATCC® CRL-2539™) mouse breast adenocarcinoma cell line was purchased from ATCC. The cells were maintained at 37 °C/5% CO₂ in Roswell Park Memorial Institute medium (RPMI-1640, LM-R1637, biosera) supplemented with 10% fetal bovine serum (FBS, FB-1001H, biosera) and 1% antibiotics (A5955, Sigma). The MCA205, mouse fibrosarcoma cell line was purchased from Millipore (SCC173, Millipore) and cultured in expansion medium consisting of Roswell Park Memorial Institute medium (RPMI-1640, LM-R1637, biosera) containing 2 mM L-glutamine (TMS-002-C, Sigma), 1 mM sodium pyruvate (TMS-005-C, Sigma), 10% fetal bovine serum (FBS, FB-1001H, biosera), 1x non-essential amino acids (TMS-001-C, Sigma), 1% antibiotics (A5955, Sigma) and 1x β-mercaptoethanol (ES-007-E, Sigma).

Syngeneic tumor models. A breast orthotopic syngeneic tumor model was generated by implantation of $5 \times 10^4$ 4T1 cancer cells in 40 µl of serum-free medium into the third mammary fat pad of 6-8-week-old BALB/c female mice. A fibrosarcoma syngeneic tumor model was generated by implantation of $2.5 \times 10^5$ MCA205 cells in 50 µL of serum-free medium into the flank of 6-8 week old C57BL/6 male mice. Mice were purchased from the Cyprus Institute of Neurology and Genetics and all in vivo experiments were conducted in accordance with the animal welfare regulations and guidelines of the Republic of Cyprus and the European Union (European Directive 2010/63/EE and Cyprus Legislation for the protection and welfare of animals, Laws 1994-2013) under a license acquired and approved (No CY/EXP/PR.L2/2018, CY/EXP/PR.L14/2019, CY/EXP/PR.L15/2019, CY/EXP/PR.L03/2020) by the Cyprus Veterinary Services, the Cyprus national authority for monitoring animal research.
Drugs and reagents. Doxil (Pegylated liposomal doxorubicin, Janssen Pharmaceuticals) was purchased as already made solution (2 mg/ml). The immune checkpoint inhibitor (ICI) mouse monoclonal aPDL1 was purchased from BioXCell.

Interstitial Fluid Pressure IFP. Before the end of the experiment, animals were anesthetized by i.p. injection of Avertin (200 mg/kg) and interstitial fluid pressure (IFP) was measured using the wick-in-needle technique [1].

Tumor Opening experiment for quantification of growth-induced stress. Upon excision, we cut the tumors along their longest axis at approximately 80% of their thickness. We then allowed tumors to relax and measured the formed distance (tumor opening) between the two hemispheres [2]. Representative image of experimental procedure is given in Supplementary Figure S4A.

Description of the mathematical model
The mathematical model has been described in detail in our previous work [3, 4] for the study of metronomic chemotherapy and has been extended here to account for the delivery of nanomedicine. Nanomedicines have been modeled as nanocarriers that contain molecules of chemotherapy and are released at a constant release rate. Nanoparticles and chemotherapy can extravasate from the pores of the vessel walls, diffuse into the tumor interstitial space, bind to cancer cells and get internalized by the cells [5, 6]. The equations for drug delivery that are being solved by the model for delivery of chemotherapy have the form:

\[
\begin{align*}
\frac{\partial c_f}{\partial t} + \nabla \cdot \left( c_f \mathbf{v} \right) &= D_f \nabla^2 c_f + Q_{sta} - \frac{k_{on} c_c c_f}{\Phi} + k_{off} c_b, \\
\frac{\partial c_b}{\partial t} + \nabla \cdot \left( c_b \mathbf{v} \right) &= \frac{k_{on} c_c c_f}{\Phi} - k_{off} c_b - k_{int} c_{int}, \\
\frac{\partial c_{int}}{\partial t} + \nabla \cdot \left( c_{int} \mathbf{v} \right) &= k_{int} c_b,
\end{align*}
\]

where we assumed that chemotherapy affects the growth of the tumor by killing cancer cells but has no effect on endothelial cells and the tumor vascular density. The chemotherapeutic agent can
exist in three distinct states: free to travel in the interstitial space \((c_f)\), bind to cancer cells \((c_b)\), and internalized by cells \((c_{int})\). \(D_f\) is the diffusion coefficient of the drug, \(k_{on}\), \(k_{off}\) and \(k_{int}\) are the association (binding), dissociation and internalization rate constants of the drug to cancer cells, \(c_e\) is the concentration of cell surface receptors, \(\Phi\) is the volume fraction of tumor accessible to the drug, \(\mathbf{v}^s\) and \(\mathbf{v}^f\) are the velocities of solid and fluid phase respectively and \(Q_{sta}\) is the amount of drug that extravasates from the vessels into the tumor and is given later by Eq. 7.

Supplementary Table S1 depicts all model parameters employed by the model as well as their values.

In the case of the delivery of a nanoparticle carrier \((c_n)\) containing the chemotherapy, the drug transport equations take the form:

(A) Delivery of nanomedicine (without binding)

\[
\frac{\partial c_n}{\partial t} + \nabla \cdot \left( c_n \mathbf{v}^f \right) = D_n \nabla^2 c_n + Q_{sta} - k_{rel} c_n
\]

\[
\frac{\partial c_f}{\partial t} + \nabla \cdot \left( c_f \mathbf{v}^f \right) = D_f \nabla^2 c_f + a k_{rel} c_n - k_{int} c_f
\]

\[
\frac{\partial c_{int}}{\partial t} = k_{int} c_f
\]

(B) Delivery of nanomedicine (with binding)

\[
\frac{\partial c_n}{\partial t} + \nabla \cdot \left( c_n \mathbf{v}^f \right) = D_n \nabla^2 c_n + Q_{sta} - k_{rel} c_n - k_{on} c_n + k_{off} c_{nb}
\]

\[
\frac{\partial c_{nb}}{\partial t} = k_{on} c_n - k_{rel} c_{nb} - k_{off} c_{nb}
\]

\[
\frac{\partial c_f}{\partial t} + \nabla \cdot \left( c_f \mathbf{v}^f \right) = D_f \nabla^2 c_f + a k_{rel} (c_n + c_{nb}) - k_{int} c_f - k_{off} c_{nb}
\]

\[
\frac{\partial c_{int}}{\partial t} = k_{int} c_f
\]

where \(c_{nb}\) is the concentration of nanoparticles that binds to cancer cells, \(D_n\) and \(D_f\) are the diffusion coefficients of the nanoparticle and chemotherapy, respectively, in the tumor interstitial space, \(k_{rel}\) is the release rate constant of chemotherapy form the nanoparticle and \(\alpha\) is the number of chemotherapy molecules contained in the nanocarrier. The interstitial fluid velocity \(\mathbf{v}^f\) depends on
the interstitial hydraulic conductivity \( k_{th} \) and the interstitial fluid pressure gradient, given by Darcy’s law:

\[
v^f = -k_{th} \nabla p_i
\]  

Combining Darcy’s law with the continuity equation \((\nabla \cdot v^f = Q)\) yields the steady-state fluid transport model [7]:

\[
-k_{th} \nabla^2 p_i = Q
\]

where \( Q \) denotes the fluid flux entering from the blood vessels into the tumor or the surrounding normal tissue minus the fluid flux exiting through lymphatic vessels [7]:

\[
Q = L_p S_v (p_v - p_i) - L_{pl} S_{vl} (p_i - p_l)
\]

where \( L_p, S_v \) and \( p_v \) are the hydraulic conductivity, vascular density and vascular pressure, respectively, \( L_{pl}, S_{vl} \) and \( p_l \) are the corresponding quantities for lymphatic vessels, and \( p_i \) is the interstitial fluid pressure.

The term \( Q_{sta} \) on the right hand side of Eqs. (1-3) denotes the transport of the drug across the tumor vessel wall and is given by Starling’s approximation [8]:

\[
Q_{sta} = P \cdot S_v (C_{iv} - c_n) + L_p S_v (p_v - p_i) (1 - \sigma_f) C_{iv}
\]

\( C_{iv} \) is the vascular concentration of the nanoparticle and is taken to be \( C_{iv} = \exp(- (t-t_0)/k_d) \) describing a bolus injection, with \( t_0 \) the time of drug injection and \( k_d \) the blood circulation decay of nanoparticle, \( \sigma_f \) is the reflection coefficient and \( P \) is the vascular permeability. The parameters \( P, L_p \) and \( \sigma_f \) that govern the transvascular transport (i.e., extravasation rate) of the nanoparticles across the tumor vessel walls are calculated as a function of the size of the pores of the vessel walls
and the size of the nanoparticle accounting for steric and hydrodynamic interactions among the particles and the vessel walls by [9]:

\[ P = \frac{\gamma HD_0}{L} \]  

(8)

where \( \gamma \) is the fraction of vessel wall surface area occupied by pores, \( H \) is a parameter describing hydrodynamic and steric interactions that hinder the diffusive transport of the nanoparticle through the pores of the vessel wall, \( L \) is the thickness of the vessel wall and \( D_0 \) is the diffusion coefficient of a particle in free solution at 310K, given by the Stokes-Einstein relationship:

\[ D_0 = \frac{K_b T}{6\pi \eta r_s} \],

(9)

where \( K_b \) is the Boltzmann constant, \( T \) is temperature, \( \eta \) is the viscosity of blood and \( r_s \) the radius of the nanoparticle.

The hydraulic conductivity of the vessel wall was calculated from the expression [9]:

\[ L_p = \frac{\gamma \alpha^2}{8\eta L} \],

(10)

where \( \alpha \) is the pore radius.

The reflection coefficient is given by the equation [9]:

\[ \sigma_f = 1 - W \]

(11)

where \( W \) describes hydrodynamic and steric interactions that hinder the convective transport of the nanoparticle through the pores of the vessel walls.

Ignoring electrostatic interactions, \( H \) and \( W \) are reduced to [9]:
\[ H = \frac{6\pi F}{K_t} \]  
\[ W = \frac{F(2 - F)K_s}{2K_t} \]

where \( F \) is the partition coefficient \([9]\):

\[ F = (1 - \lambda)^2 \]

and \( \lambda \) is the ratio of the drug size to the vessel wall pore size.

The coefficients \( K_s \) and \( K_t \) are determined by:

\[
\left( \frac{K_t}{K_s} \right) = \frac{9}{4}\pi^2 \sqrt{2} \left( 1 - \lambda \right)^{5/2} \left[ 1 + \sum_{n=1}^{2} \left( a_n \right) (1 - \lambda)^n \right] + \sum_{n=0}^{4} \left( b_{n+3} \right) \lambda^n
\]

Cancer cell proliferation

The mathematical model accounts for the growth of a spherical tumor surrounded by normal tissue. To calculate the growth rate of the tumor we took into account three types of cancer cells: non-stem cancer cell (CC), stem-cell-like cancer cell (CSC) and induced cancer cell (ICC) proliferation, as well as tumor oxygenation \([10-14]\). In particular, to calculate the growth stretch ratio \( \lambda_g \) we used the expression

\[
\frac{d\lambda_g}{dt} = \frac{1}{3} \left( \frac{T}{T_{tot}} S_T^c + \frac{C_{sc}}{T_{tot}} S_{sc}^c + \frac{I}{T_{tot}} S_I^c \right) \lambda_g
\]

where \( T \) is the CC population, \( C_{sc} \) is the CSC population, \( I \) is the ICC population, \( T_{tot} \) is the total density of cells given by the sum of the three populations, and \( S_T^c \), \( S_{sc}^c \) and \( S_I^c \) are the proliferation/degradation rates of CCs, CSCs and ICCs, respectively calculated as a function of oxygen \([3]\).
Cancer cells, immune cells and Tumor Associated Macrophages (TAMs) population balance

Four types of immune cells are considered: the natural killer (NK) cells, the CD8\(^{+}\) T-cells, the conventional CD4\(^{+}\) T-cells and the regulatory CD4\(^{+}\) T cells (Treg) [15-17]. The system of equations accounts for the recruitment rates of the immune cells, their inactivation by cancer cells, the inhibitory role of Treg cells as well as their death rate and interaction with cancer cells. Also, we account for two different types of TAMs, M1-like and M2-like.

The equations that describe the conservation of cancer cells, immune cells and TAMs are given below:

\[
\frac{\partial T}{\partial t} = \nabla \cdot (D_{\text{cell}} \nabla T) + GS_{\text{fr}} T - cNT - D + p_{CT} C_{sc} + p_{IT} I - (p_{TC} + p_{IT}) T - \lambda_{M1} M_{1} T
\]

\[
\frac{\partial C_{\text{csc}}}{\partial t} = \nabla \cdot (D_{\text{cell}} \nabla C_{sc}) + \alpha_{csc} GS_{\text{fr}_{csc}} C_{sc} - c_{csc} NC_{sc} - D_{csc} + p_{Tc} T + p_{IC} I - (p_{CT} + p_{CI}) C_{sc}
\]

\[
\frac{\partial I}{\partial t} = \nabla \cdot (D_{\text{cell}} \nabla I) + \alpha_{I} GS_{\text{fr}_{I}} I - c_{I} NI - D_{I} + p_{IT} T + p_{CT} C_{sc} - (p_{IT} + p_{IC}) I
\]

\[
\frac{\partial N}{\partial t} = \sigma_{nk} - f_{NK} N + \frac{g_{sk} T^{2}}{h + T^{2}} N - p_{im} NT - \lambda_{\text{reg}} T_{\text{reg}} N - \lambda_{M2} M_{2} N
\]

\[
\frac{\partial L}{\partial t} = -m_{T8} L + \frac{j_{T8} D^{2}}{k_{im} + D^{2}} L - qLT + (r_{N} N + r_{C_{d4}} C_{d4}) T - \lambda_{\text{reg}} T_{\text{reg}} L - \lambda_{M2} M_{2} L
\]

\[
\frac{\partial C_{d4}}{\partial t} = s_{C_{d4}} + re_{C_{d4}} C_{d4} \left(1 - \frac{C_{d4}}{C_{d4,\text{max}}}ight) - \mu_{C_{d4}} C_{d4}
\]

\[
\frac{\partial T_{\text{reg}}}{\partial t} = g_{\text{reg}} T_{\text{reg}} - m_{\text{reg}} T_{\text{reg}}
\]

\[
\frac{\partial M_{1}}{\partial t} = g_{m1} M_{1} - m_{m1} M_{1}
\]

\[
\frac{\partial M_{2}}{\partial t} = g_{m2} M_{2} - m_{m2} M_{2} + r_{\text{vegf},M_{2}} C_{\text{vegf}} M_{2}
\]

where \(N\) is the density of NK cells, \(L\) is density of tumor-specific CD8\(^{+}\) T-cells, \(C_{d4}\) is the density of conventional CD4\(^{+}\) T-cells, \(T_{\text{reg}}\) is the density of the regulatory CD4\(^{+}\) T cells, \(M_{1}\) the density of M1-like TAMs cells, \(M_{2}\) the density of M2-like TAMs cells and \(D_{\text{cell}}\) is the cancer cell diffusion coefficient. In the equations above all cell densities are a function of position and time, and the migration of different cell types relative to the tissue is neglected. \(G\) describes the proliferation of CCs, CSCs and ICCs as a function of oxygen and \(S_{f}\) is an expression that accounts for the fraction...
of cells surviving drug treatment. \( c \) and \( D \) are the fractions of tumor cells killed by NK and CD8\(^+\) T-cells, respectively. For the coefficients of the proliferation rates of CSCs and ICCs, i.e., \( \alpha_{csc} \) and \( \alpha_t \), respectively, we assume that for normal oxygen levels they are equal to one so that all cancer cell types have the same proliferation as that of CCs. In hypoxic conditions, however, the proliferation of cancer cells with a stem-like phenotype increases. Thus, we assume that their proliferation increases inversely proportional to the oxygen concentration, so that as oxygen concentration approaches zero, the proliferation rates are twice as much as the rate in normal oxygen [18]. For the parameters \( c_{csc}, D_{csc}, c_I, \) and \( D_I \) that describe the killing potential of immune cells on CSCs and ICCs, we assume that they are more resistant in interactions with immune cells. According to experimental data [19], the cytotoxicity of CD8\(^+\) T-cells against CSCs is taken to be 7-fold lower than that of CCs. As a result, the parameters that describe the killing of CSCs by immune cells are assumed to be the same as for the CCs but multiplied by a factor of 0.14. The rates of transfer of cancer cells from a type \( i \) to a type \( j \) are described by \( p_{ij} \) and their values were determined in [14]. Additionally, the parameter \( \lambda_{MI} \) denotes the tumoricidal effect of M1-like TAMs in cancer cells according to previous study [20]. \( f_{NK}, m_{TS} \) and \( m_{reg} \) are death rates of NK cells, CD8\(^+\) T-cells and Treg cells respectively, \( g_{NK}, j_{TS} \) and \( g_{reg} \) are recruitment rates of immune cells, \( p_{im} \) and \( q \) are inactivation rates of immune cells by CCs, \( \sigma_{nk} \) is constant source of NK cells, \( r_N \) is the rate at which tumor-specific CD8\(^+\) T-cells are stimulated to be produced as a result of tumor cells killed by NK cells and \( \lambda_{reg} \) is the inhibition term of NK cells and CD8\(^+\) T-cells from Treg cells. Under anoxic conditions we used the lowest value for the activity of NK cells and CD8\(^+\) T-cells reported in de Pillis et al. [15], which increased linearly to the highest value for normal oxygen conditions. The values of \( f_{NK} \) and \( m_{TS} \) are modified to depend on oxygen levels. According to experimental data [21], a 40 times decrease in oxygen concentration (from 20% to 0.5%) doubled the apoptotic rate of immune cells. Additionally \( s_{CD4} \) is the source of conventional CD4\(^+\) T-cells, \( \mu_{Cd4} \) is the natural death rate of conventional CD4\(^+\) T-cells, \( r_{Cd4} \) is the growth rate of conventional CD4\(^+\) T-cells and \( C_{d4, max} \) is the maximum conventional CD4\(^+\) T-cells population [22, 23]. \( r_{Cd4} \) is the stimulation rate of CD8\(^+\) T-cells by conventional CD4\(^+\) T-cells as mentioned previously [24-26]. The source term of conventional CD4\(^+\) T-cells \( s_{CD4} \) will depend on oxygen concentration, as according to previous studies under hypoxic conditions it decreased 8 times [27]. Furthermore, a decrease of M2-like TAMs resulted in higher numbers of CD8\(^+\) T-cells and NK cells, while conventional CD4\(^+\) T-cells were not affected according to experimental data [28] and
these observations are described by the parameter \( \lambda_{M2} \). \( g_{M1} \) and \( g_{M2} \) are the production rates of M1-like and M2-like TAMs, which depend on oxygen levels according to previous studies [28-30] showing that a decrease in hypoxia skewing TAMs polarization away from the M2- to M1-like phenotype. According to previous studies TAMs are associated with VEGF expression [28, 31, 32]. Specifically, VEGF-A overexpression correlated with higher numbers of M2-like TAMs \( (r_{C_{vegf,M2}}) \). The range of values of the model parameters are summarized in Supplementary Table S1. The above equations are rendered dimensionless by dividing the number of cells per finite element node by the initial number of cancer cells, \( T_0=5\times10^2 \) cells. The initial population of cancer cells was taken to be: 98% CCs, 1% CSCs and 1% ICCs [33].

The dependence of cancer cell proliferation on the local oxygen concentration, \( G \), is assumed to follow a Michaelis-Menten kinetic and has the form [34, 35]:

\[
G = \frac{k_1 c_{ox}}{k_2 + c_{ox}},
\]

(18)

where \( k_1 \) and \( k_2 \) are growth rate parameters and \( c_{ox} \) is the oxygen concentration.

The parameter \( D \) denotes the fractional cell kill of tumor cells by CD8\(^+\) T-cells and given by equation [15, 36]:

\[
D = d_{im} \left( \frac{L}{T} \right)^{\lambda_{im}} \frac{L}{T} \left( \frac{L}{T} \right)^{-\lambda_{im}} T,
\]

(19)

where \( d_{im} \) is the saturation level of fractional tumor cell kill by CD8\(^+\) T-cells, \( s \) is steepness coefficient of the tumor-CD8\(^+\) T-cells competition term and \( \lambda_{im} \) the exponent of fractional cell kill by CD8\(^+\) T-cells.

To account for the effect of drug delivery on growth, the surviving fraction of cells \( S_{fr} \) is included in Eq. (17), so that in the absence of drugs \( S_{fr} \) equals unity. The fraction of surviving cells with respect to drug concentration has been previously measured experimentally for doxorubicin [37], and the results were fitted to an exponential expression as a function of the internalized chemotherapy concentration \( c_{int} \), i.e.,
\[ S_{fr} = 2^*(\exp(-\omega c_{int})-0.5) \]  

where \( \omega \) is a fitting parameter which depends on the potency of the drug and it is calculated by fitting Eq. (20) to experimental data.

**Biphasic formulation of the tumor’s mechanical behavior**

Tumor growth is modelled based on principles from continuum mechanics and particularly the multiplicative decomposition of the deformation gradient tensor \( (F) \). The kinematics of the tumor are decomposed into two components, the growth component \( (F_g) \) that accounts for the growth of the tumor and the elastic component \( (F_e) \) that accounts for mechanical interactions of the tumor with the surrounding normal tissue [38, 39]:

\[ F = F_e F_g, \]  

(21)

The growth component is set to be homogenous and isotropic [40-42]

\[ F_g = \lambda_g I, \]  

(22)

where \( \lambda_g \) is defined in Eq. (16). The elastic component \( F_e \) of the deformation gradient tensor is determined from Eq. (21) as

\[ F_e = F F_g^{-1}. \]  

(23)

The tumor is assumed to be composed of a solid (cancer and immune cells and extracellular matrix) and an interstitial fluid phase. The conservation of the tumor’s solid and fluid phase is given by the mass balance equations [10, 13]:

\[ \frac{\partial \Phi^c}{\partial t} + \nabla \cdot (v^c \Phi^c) = \frac{T}{T_{tot}} S^c + \frac{C_{sc}}{T_{tot}} S_{Csc} + \frac{I}{T_{tot}} S^f, \]  

(24)

\[ \frac{\partial \Phi^f}{\partial t} + \nabla \cdot (v^f \Phi^f) = Q, \]  

(25)

where \( \Phi^c \) and \( \Phi^f \) are the volume fractions of the solid and fluid phases, respectively.

According to the biphasic theory for soft tissues [43], the total stress tensor \( \sigma_{tot} \) is the sum of the fluid phase stress tensor \( \sigma^f = -p I \) and the solid phase stress tensor \( \sigma^s \). As a result, the stress balance is written as:
\[ \nabla \cdot \mathbf{\sigma}_{\text{tot}} = 0 \implies \nabla \cdot (\mathbf{\sigma}^s - P_I \mathbf{I}) = 0, \] (26)

where the Cauchy stress tensor of the solid phase \( \mathbf{\sigma}^s \) is given by [44]:

\[ \mathbf{\sigma}^s = J_e^{-1} \mathbf{F}_e \frac{\partial W}{\partial \mathbf{F}^T_e}, \] (27)

**Functional vascular density calculation**

Vascular density is affected by the decrease in the vessel diameter caused by increased number of cancer cells [45]. Assuming that the number of cancer cells does not affect the number or length of vessels, but only their diameter, the change in vascular density due to vessel compression is expressed as:

\[ S_v = \frac{d}{d_0} S_v^0 \rho_v^{EC}, \] (28)

where \( S_v^0 \) is the vascular density of the host tissue, \( S_v^0 = 70 \text{ cm}^{-1} \) and \( \rho_v^{EC} \) is the density of endothelial cells which is given below. Fitting experimental data [6, 45, 46] to a mathematical equation, an expression for degree of vessel compression (i.e., \( d/d_0 \)) as a function of cancer cell density and solid stress levels was estimated.

**Oxygen transport equation**

A convection-diffusion-reaction type equation is employed for the calculation of the rate of change of oxygen in the tumor. The reaction term is related to the oxygen transferred from the vessels to the tumor, minus the amount of oxygen consumed by cells [10, 11], i.e.,

\[
\frac{\partial c_{ox}}{\partial t} + \nabla \cdot (c_{ox} \mathbf{v}^f) = D_{ox} \nabla^2 c_{ox} - \frac{A_{ox} c_{ox}}{c_{ox} + k_{ox}} \Phi^C + P_{er} S_v (C_{iox} - c_{ox}),
\] (29)

where \( c_{ox} \) is the oxygen concentration, \( D_{ox} \) is the diffusion coefficient of oxygen in the interstitial space, \( A_{ox} \) and \( k_{ox} \) are oxygen uptake parameters, \( P_{er} \) is the vascular permeability of oxygen that describes diffusion across the tumor vessel wall and \( C_{iox} \) is the oxygen concentration in the vessels.
**Endothelial cell transport equation**

The flux of endothelial cells is given by the equation [47]:

\[
\frac{\partial \hat{e}}{\partial t} = \nabla \cdot (D_{EC}(a_1, a_2)\nabla \hat{e} - x_n\hat{e}H(1-\hat{e})C_{vegf}^0\nabla \hat{C}_{vegf} - W_{Se}x_n\hat{e}H(1-\hat{e})C_S^0\nabla \hat{C}_S) + \\
\frac{1}{\hat{e}_0}(\lambda_1 C_{vegf}^0 e_0 \hat{C}_{vegf} \hat{e} + \lambda_2 C_{vegf}^0 e_0 \hat{C}_{vegf} \hat{e} H(1-\hat{e}) - (\lambda_3 e_0 \hat{e} + \lambda_4 e_0 \hat{e}) \hat{e})
\]

(30)

Endothelial cell proliferation is based on VEGF and CXCL12 concentration as well as endothelial cell density. \( \hat{e} \) is the dimensionless endothelial cell density. \( C_{vegf}^0 \) and \( C_{vegf}^0 \) are dimensionless and reference VEGF concentrations. Endothelial cell diffusion coefficient depends on Ang1 and Ang2: \( D_{EC}(a_1, a_2) = D_e(a_1 + s_1 a_1 a_2)^b \) with \( a \) and \( b \) to be unity [48]. \( \chi_n \) is a chemotactic term and \( W_{Se} \) is a weighting function describing the contribution of VEGF and CXCL12 on endothelial cell transport. The dimensionless concentration of the endothelial cells is calculated by division with the reference concentration \( \hat{e} = \frac{e}{e_0} \). Loss terms describing killing of endothelial cells are also included. The parameters \( \lambda_1, \lambda_2, \lambda_3 \) and \( \lambda_4 \) are constant positive parameters.

**Pericytes transport equation**

Two populations/phenotypes of pericytes are considered: pericytes that are tightly associated with endothelial cells and assumed to be immotile and pericytes that are dissociated from endothelial cells and can be motile. Production rates of both phenotypes depends on PDGF-B concentration as well as on their own concentrations.

**Immotile pericytes transport equation**

The pericytes density is given by the equation [49, 50]:

\[
\frac{\partial p_{cim}}{\partial t} = \beta_{pc} \frac{p_{cim}}{1 + p_c / p_c^0} \frac{p_b H(\lambda_{p_b} p_b - c_{p_b})}{p_b + \alpha_{p1}} - \mu_{pc} \frac{a_2 H(a_2 - \alpha_{p2})}{a_2 + \alpha_{p2}} p_{cim} + \alpha_{p4} (p_{cimmax} - p_{cim}),
\]

(31)

where \( p_c \) is the total pericytes density \( (p_c = p_{cim} + p_{cm}) \), \( p_{cim}^0 \) is the pericyte reference value, \( p_b \) is the PDGF-B concentration, \( p_{cimmax} \) is the carrying capacity of the immotile pericyte density, \( \beta_{pc}, \lambda_{p_b}, c_{p_b}, \alpha_{p1}, \alpha_{p2}, \alpha_{p3}, \alpha_{p4}, \mu_{pc} \) are constant positive parameters.
Motile pericytes cells transport equation

The motile pericyte density is given by the equation [49, 50],

\[
\frac{\partial p_{cm}}{\partial t} = \nabla \cdot (D_{pc} \nabla p_{cm}) - \nabla \cdot (k_{pc} p_{cm} \nabla p_{b}) - a_{p,4} (p_{c,\text{max}} - p_{c,\text{im}}) + \beta_{pc} \frac{p_{cm}}{1 + p_{c} / p_{c}^{0}} p_{b} \frac{H(\lambda_{p,c} - c_{p,c})}{p_{b} + \alpha_{p,c}},
\]

where \( k_{pc} \) is a chemotactic constant, \( D_{pc} \) is the diffusion coefficient of motile pericytes and \( \mu_{pc} \) is a constant positive parameter.

VEGF transport equation

VEGF concentration is determined by diffusion, production from cancer cells under hypoxic conditions and binding to endothelial cells receptors [47]. VEGF concentration is governed by the equation [47]:

\[
\frac{\partial \tilde{C}_{\text{vegf}}}{\partial t} = \nabla \cdot (D_{\text{VEGF}} \nabla \tilde{C}_{\text{vegf}}) + \frac{\lambda_{\text{vegf}}}{C_{\text{vegf}}^{0}} e^{G_{a}(\tilde{c}_{\text{o}x})} - (\lambda_{1} \tilde{e} + \lambda_{2} \tilde{e} + \lambda_{3}) \tilde{C}_{\text{vegf}} - \lambda_{\text{CD4,C,vegf}} \tilde{C}_{\text{vegf}} \tilde{C}_{\text{vegf}},
\]

Where \( \tilde{C}_{\text{vegf}} \) is the dimensionless VEGF concentration calculated with division with a reference value \( \tilde{C}_{\text{vegf}}^{0} \) and \( \tilde{c}_{\text{o}x} \) is the dimensionless oxygen concentration normalized as: \( \tilde{c}_{\text{o}x} = \frac{c_{\text{o}x}}{c_{\text{o}x}^{0}} \).

VEGF is assumed to be produced by cancer cells only and its production is enhanced under hypoxic conditions as described by the oxygen tension term \( G_{a} \) [47].

\[
G_{a}(\tilde{c}_{\text{o}x}) = \begin{cases} 
3\tilde{c}_{\text{o}x} & \text{for } 0 < \tilde{c}_{\text{o}x} < 0.5 \ (\text{hypoxia}) \\
2 \tilde{c}_{\text{o}x} & \text{for } 0.5 < \tilde{c}_{\text{o}x} < 1 \ (\text{normoxia}) \\
\tilde{c}_{\text{o}x} & \text{for } 1 < \tilde{c}_{\text{o}x} \ (\text{hyperoxia}) 
\end{cases}
\]
VEGF becomes unavailable due to binding to endothelial cells VEGF receptors and it can also diffuse in the tumor with a diffusion coefficient $D_{VEGF}$. $\lambda_{10}$, $\lambda_{11}$, $\lambda_{12}$ and $\lambda_{13}$ are positive constants. Additionally, knockout of conventional CD4$^+$ T cells resulted in overexpression of VEGF ($\lambda_{CD4,Cvegf}$) and not significant differences in Ang1-Ang2 [24].

**CXCL12 transport equation**

The stromal cell derived factor 1 (SDF1α) is also known as C-X-C motif chemokine 12 (CXCL12). We suggest in the model that VEGF released by hypoxic cancer cells up-regulates CXCL12 from cancer cells and that CXCL12 is also produced by endothelial cells in a VEGF dependent manner [51]. Therefore, CXCL12 is produced by both cancer cells and endothelial cells and it is also up-regulated by hypoxia and VEGF [51]. The transport of CXCL12 is governed by:

$$\frac{\partial \hat{C}_S}{\partial t} = \frac{\lambda_{10}}{C_s^0} G_a (\hat{c}_o) T + \frac{\lambda_{13}}{C_s^0} C_v^0 \hat{C}_v H (1 - \hat{e}) - \lambda_{13} \hat{C}_S$$

(34)

where $\lambda_{10}$ and $\lambda_{13}$ are positive parameters. The dimensionless CXCL12 concentration is given by division with a reference concentration $\hat{C}_S = \frac{C_s}{C_S^0}$.

**PDGF-B transport equation**

PDGF-B was assumed to be produced by endothelial cells and binds to pericytes [52]. PDGF-B concentration is governed by the equation [53]:

$$\frac{\partial \hat{p}_b}{\partial t} = D_{pb} \nabla^2 \hat{p}_b + \beta_{pb} \hat{e} - \mu_{pb} \hat{p}_b - \gamma_{pb} \hat{p}_b \hat{p}_c$$

(35)

where $\beta_{pd}$, $\mu_{pb}$ and $\gamma_{pb}$ are positive parameters, $D_{pb}$ is the PDGF-B diffusion coefficient.
**Ang1 and Ang2 transport equations**

Ang1 is assumed to be produced by pericytes and Ang2 by endothelial cells, respectively. Their production is enhanced by hypoxia based on VEGF levels [48]. Angiopoietin 1 (Ang1, \( \alpha_1 \)) and angiopoietin 2 (Ang2, \( \alpha_2 \)) are up-regulated by hypoxia and produced by endothelial cells.

\[
\frac{\partial \hat{a}_1}{\partial t} = \frac{b_1}{a_0} p_c + \mu_1(1 - \hat{a}_1)
\]

(36)

\[
\frac{\partial \hat{a}_2}{\partial t} = \frac{b_2}{a_2} G_a (\hat{c}_{ox}) \hat{e} e_0 - \mu_2 \hat{a}_2
\]

(37)

where \( b_1, b_2, \mu_1 \) and \( \mu_2 \) are positive constants. The dimensionless Ang1 and Ang2 are given by division with a reference concentration \( \hat{a}_1 = \frac{a_1}{a_1^0}, \hat{a}_2 = \frac{a_2}{a_2^0} \). The oxygen tension term \( G_a \) is the same as used for VEGF and CXCL12. For the simplicity of the equations, we neglect diffusion of Ang1 and Ang2 and binding to specific Tie receptors [54, 55].
Supplementary Table S1. Parameters and their values used in the model

| Parameter | Description | Value | Reference |
|-----------|-------------|-------|-----------|
| $\mu$     | shear modulus | 5.00 kPa for host tissue; 10.40 kPa for tumor | [56-58] |
| $k$       | bulk modulus | 6.67 kPa for host tissue; $10.40 \times 10^7$ kPa for tumor | [56-58] |
| $k_{th}$  | hydraulic conductivity | $6.5 \times 10^{-10}$ m²·Pa⁻¹·day⁻¹ | [58] |
| $C_{ox}$  | initial oxygen concentration | 0.2 mol·m⁻³ | [59] |
| $D_{ox}$  | oxygen diffusion coefficient | $1.55 \times 10^{-4}$ m²·day⁻¹ | [11] |
| $A_{ox}$  | oxygen uptake | 2,200 mol·m⁻³·day⁻¹ | [11, 59] |
| $k_{ox}$  | oxygen uptake | 0.00464 mol·m⁻³ | [11, 59] |
| $k_2$     | growth rate parameter | 0.0083 mol·m⁻³ | [59] |
| $c_e$     | receptor concentration | 0.01 mol·m⁻³ | [60, 61] |
| $\phi$    | volume fraction of tumor accessible to drug | 0.06 | [60, 61] |
| $k_{on}$  | binding rate | $1.296 \times 10^6$ day⁻¹ | [60, 61] |
| $k_{off}$ | dissociation rate | 691.2 day⁻¹ | [60, 61] |
| $k_{int}$ | internalization rate | 3.7 day⁻¹ | [60, 61] |
| $D_f$     | chemotherapy diffusion coefficient | $8.64 \times 10^{-6}$ m²·day⁻¹ | [62] |
| $\omega$  | cancer cell survival constant | 3.95 m³/mol camptothecin; 0.6603 m³/mol doxorubicin | [63, 64] |
| $a_{esc}$ | stem-cell-like cell growth multiplier | range **: 1-2 [-] | [17] |
| $a_I$     | induced cancer cell growth multiplier | range **: 1-2 [-] | --- |
| $k_d$     | blood circulation decay | 0.417 day⁻¹ | [65] |
| Symbol | Description                                           | Value                  | Reference |
|--------|-------------------------------------------------------|------------------------|-----------|
| $L_{vw}$ | vessel wall thickness                                 | $5 \times 10^{-6}$ m    | [66]      |
| $\eta$ | water viscosity at 310K                               | $7 \times 10^{-4}$ Pa·s | [66]      |
| $T_{\text{abs}}$ | absolute temperature                                | 310 K                  | ---       |
| $\gamma$ | fraction of vessel wall surface area occupied by pores | $1 \times 10^{-5}$ [-]  | [65]      |
| $c$    | fractional tumor cell kill by NK cells                | range $^*$: $3.23 \times 10^{-7}$ - $3.23 \times 10^{-6}$ cell$^{-1}$·day$^{-1}$ | [67] |
| $d_{im}$ | fractional tumor cell kill by CD8$^+$ T-cells         | range $^*$: 1.43 – 7.15 day$^{-1}$ | [67] |
| $\lambda_{im}$ | exponent of fractional cell kill by CD8$^+$ T-cells   | 1.36 [-]               | [67]      |
| $s$    | steepness coefficient of the tumor-CD8$^+$ T-cells competition term | 2.73 [-]              | [67]      |
| $\sigma_{nk}$ | constant source of NK cells                          | $1.3 \times 10^4$ cells·day$^{-1}$ | [67] |
| $f_{\text{Nk}}$ | death rate of NK cells                                | range $^{**}$: 0.0412 - 0.0814 day$^{-1}$ | [67] |
| $m_{T8}$ | death rate of CD8$^+$ T-cells                         | range $^{**}$: 0.02 - 0.04 day$^{-1}$ | [67] |
| $m_{\text{reg}}$ | death rate of Treg cells                             | 0.02 day$^{-1}$        | [16]      |
| $g_{\text{NK}}$ | recruitment rate of NK cells                         | initial$^{***}$: 0.025 day$^{-1}$ | [67] |
| $j_{T8}$ | recruitment rate of CD8$^+$ T-cells                   | initial$^{***}$: 0.0375 day$^{-1}$ | [67] |
| $g_{\text{reg}}$ | recruitment rate of Treg cells                       | initial$^{***}$: 0.0375 day$^{-1}$ | [16] |
| Parameter | Description | Value | Reference |
|-----------|-------------|-------|-----------|
| $h$       | steepness coefficient of NK cell recruitment curve | $2.02 \times 10^7 \text{cell}^2$ | [67] |
| $p_{im}$  | inactivation rate of NK cells | $1 \times 10^{-7} \text{cell}^{-1}\cdot\text{day}^{-1}$ | [67] |
| $k_{im}$  | steepness coefficient of CD8$^+$ T-cells recruitment curve | $2.02 \times 10^7 \text{cell}^2$ | [67] |
| $q$       | inactivation rate of CD8$^+$ T-cells | $3.42 \times 10^{-10} \text{cell}^{-1}\cdot\text{day}^{-1}$ | [67] |
| $r$       | stimulation rate of CD8$^+$ T-cells | $1.1 \times 10^{-7} \text{cell}^{-1}\cdot\text{day}^{-1}$ | [67] |
| $\lambda_{reg}$ | inhibition term of NK cells and CD8$^+$ T-cells from Treg cells | $100 \text{cell}^{-1}\cdot\text{day}^{-1}$ | [16] |
| $p_{TC}$  | rate of dedifferentiation from cancer cells to stem-like-cell cancer cells | $0.55 \text{day}^{-1}$ prior treatment; $0 \text{day}^{-1}$ after application of chemotherapy | [14] |
| $p_{CT}$  | rate of transition from stem-like-cell cancer cells to cancer cells | $1 \text{day}^{-1}$ prior treatment; $0.96 \text{day}^{-1}$ after application of chemotherapy | [14] |
| $p_{CI}$  | rate of transition from stem-like-cell cancer cells to induced cancer cells | $0.58 \text{day}^{-1}$ prior treatment; $0 \text{day}^{-1}$ after application of chemotherapy | [14] |
| $p_{IC}$  | rate of transition from induced cancer cells to stem-like-cell cancer cells | $0.96 \text{day}^{-1}$ prior treatment; $0.38 \text{day}^{-1}$ after application of chemotherapy | [14] |
| $p_{TI}$ | rate of transition from cancer cells to induced cancer cells | $0.21 \text{ day}^{-1}$ prior treatment; $1 \text{ day}^{-1}$ after application of chemotherapy | [14] |
| $p_{TI}$ | rate of transition from induced cancer cells to cancer cells | $1 \text{ day}^{-1}$ prior treatment; $0.98 \text{ day}^{-1}$ after application of chemotherapy | [14] |
| $\lambda_{MI}$ | tumoricidal effect of M1-like TAMs in cancer cells | $3 \text{ s}^{-1}$ | [20] |
| $g_{MI}$ | production rate of M1-like TAMs | initial***: $0.0375 \text{ day}^{-1}$ | [28, 29] |
| $g_{M2}$ | production rate of M2-like TAMs | initial***: $0.0375 \text{ day}^{-1}$ | [28, 29] |
| $m_{MI}$ | death rate of M1-like TAMs | $0.02 \text{ day}^{-1}$ | [4] |
| $m_{M2}$ | death rate of M2-like TAMs | $0.02 \text{ day}^{-1}$ | [4] |
| $r_s$ | size of nanoparticle carrier | 20 nm CRLX101; 100 nm DOXIL | [5, 68] |
| $k_{el}$ | chemotherapy release rate | $0.3 \text{ day}^{-1}$ CRLX101; 0.181 day$^{-1}$ DOXIL | [68, 69] |
| $\alpha$ | chemotherapy molecules contained in nanocarrier | 12 CRLX101; 10000 DOXIL | [70, 71] |
| $k_d$ | blood circulation decay | $1 \text{ day}^{-1}$ | [68] |
| $D_n$ | nanoparticle diffusion coefficient | $6 \times 10^{-7} \text{ m}^2.$day$^{-1}$ CRLX101; $8.64 \times 10^{-8} \text{ m}^2.$day$^{-1}$ DOXIL | [62] |
| | | |
|---|---|---|
| $L$ | Vessel wall thickness | $5 \times 10^{-6}$ m |
| $p_v$ | Vascular pressure | 30 mmHg |
| $\eta$ | Blood viscosity | $3 \times 10^{-3}$ mmHg s |
| $\gamma$ | Fraction of vessel wall surface area occupied by pores | $5 \times 10^{-4}$ [-] |
| $a_1$ | Coefficient for $K_i$ | $-73/60$ [-] |
| $a_2$ | Coefficient for $K_i$ | $77.293/50.400$ [-] |
| $a_3$ | Coefficient for $K_i$ | $-22.5083$ [-] |
| $a_4$ | Coefficient for $K_i$ | $-5.617$ [-] |
| $a_5$ | Coefficient for $K_i$ | $-0.3363$ [-] |
| $a_6$ | Coefficient for $K_i$ | $-1.216$ [-] |
| $a_7$ | Coefficient for $K_i$ | $1.647$ [-] |
| $b_1$ | Coefficient for $K_s$ | $7/60$ [-] |
| $b_2$ | Coefficient for $K_s$ | $-2.227/50.400$ [-] |
| $b_3$ | Coefficient for $K_s$ | $4.0180$ [-] |
| $b_4$ | Coefficient for $K_s$ | $-3.9788$ [-] |
| $b_5$ | Coefficient for $K_s$ | $-1.9215$ [-] |
| $b_6$ | Coefficient for $K_s$ | $4.392$ [-] |
| $b_7$ | Coefficient for $K_s$ | $5.006$ [-] |
| $D_{VEGF}$ | VEGF diffusion coefficient | $3.1 \times 10^{-11}$ [m$^2$/s] |
| $D_{ec}$ | Endothelial cell diffusion coefficient | $1 \times 10^{-15}$ [m$^2$/s] |
| Parameter | Description | Value | Reference |
|-----------|-------------|-------|-----------|
| \(D_{pb}\) | PDGF-B diffusion coefficient | \(1.65 \times 10^{-3}\) [mm\(^2\)/h] | [50] |
| \(\beta_{pb}\) | Non-negative parameter | \(1.25 \times 10^4\) [1/h] | [50] |
| \(\gamma_{pb}\) | Non-negative parameter | \(2.5 \times 10^6\) [1/(μM.h)] | [50] |
| \(\mu_{pb}\) | Non-negative parameter | \(10^{-1}\) [1/h] | [50] |
| \(\lambda_{pb}\) | Positive parameter | 100% | [50] |
| \(c_{pb}\) | Positive parameter | \(3.33 \times 10^{-3}\) [μM] | [50] |
| \(D_{pc}\) | Diffusion coefficient of motile pericyte | \(1.65 \times 10^{-3}\) [mm\(^2\)/h] | [50] |
| \(k_{pc}\) | Pericyte chemotactic | \(10^{-1}\) [mm\(^2\)/(μM.h)] | [50] |
| \(\beta_{pc}\) | Non-negative parameter | \(1.25 \times 10^{-1}\) [1/h] | [50] |
| \(\mu_{pc}\) | Non-negative parameter | \(4.17 \times 10^{-2}\) [1/h] | [50] |
| \(\mu_{pc2}\) | Non-negative parameter | \(4.17 \times 10^{-2}\) [1/h] | [50] |
| \(a_{pc1}\) | Positive parameter | \(3.33 \times 10^{-3}\) [μM] | [50] |
| \(a_{pc2}\) | Positive parameter | \(10^{-3}\) [μM] | [50] |
| \(a_{pc3}\) | Positive parameter | \(10^{-3}\) [μM] | [50] |
| \(a_{pc4}\) | Positive parameter | \(4.17 \times 10^{-3}\) [1/h] | [50] |
| \(p_c^0\) | Reference pericyte | \(3.32 \times 10^{-8}\) [μM] | [50] |
| Symbol | Description | Value | Reference |
|--------|-------------|-------|-----------|
| $x_n$  | Chemotactic endothelial cell | $2 \times 10^{-15}$ [m$^5$/kg-s] | [47] |
| $W_{ST}$ | Weight between oxygen- CXCL12 | $1$ | [47] |
| $W_{Se}$ | Weight between VEGF- CXCL12 | $1$ | [47] |
| $C_s^0$ | Reference CXCL12 concentration | $1 \times 10^{-3}$ [g/m$^3$] | [47] |
| $C_{vegf}^0$ | Reference VEGF concentration | $1 \times 10^{-3}$ [g/cm$^3$] | [47] |
| $e^0$  | Reference value of endothelial cell | $1 \times 10^{-3}$ [g/cm$^3$] | [47] |
| $a_1^0$ | Reference $a_1$ concentration | $1 \times 10^{-3}$ [g/cm$^3$] | [48] |
| $a_2^0$ | Reference $a_2$ concentration | $1 \times 10^{-3}$ [g/cm$^3$] | [48] |
| $s_{CD4}$ | Source term of conventional CD4$^+$ T- cells | $150$ day$^{-1}$ | [22] |
| $\mu_{CD4}$ | Natural death rate of conventional CD4$^+$ T-cells | $0.02$ day$^{-1}$ | [22] |
| $r_{e_{Cd4}}$ | The growth rate of conventional CD4$^+$ T-cells | $0.03$ day$^{-1}$ | [22] |
| $r_{CD4}$ | Stimulation rate of CD8$^+$ T cells by | $1 \times 10^{-15}$ cells$^{-1}$day$^{-1}$ | (23) |
| Parameter | Description | Value | Reference |
|-----------|-------------|-------|-----------|
| $\lambda_4$ | Positive parameters | $1 \times 10^{-1} \text{ cm}^3/\text{g-s}$ | [47] |
| $\lambda_5$ | Positive parameters | $5.56 \times 10^{-7} \text{ [1/s]}$ | [47] |
| $\dot{\lambda}_{10}$ | Positive parameters | $6.8 \times 10^{-3} \text{ [1/s]}$ | [47] |
| $\lambda_{11}$ | Positive parameters | $4 \text{ [cm}^3/\text{g-s]}$ | [47] |
| $\lambda_{12}$ | Positive parameters | $4 \text{ [cm}^3/\text{g-s]}$ | [47] |
| $\lambda_{13}$ | Positive parameters | $4 \times 10^{-3} \text{ [1/s]}$ | [47] |
| $b_1$ | Positive parameters | $2280 \text{ [1/h]}$ | [48] |
| $\mu_1$ | Positive parameters | $456 \text{ [1/h]}$ | [48] |
| $\mu_2$ | Positive parameters | $456 \text{ [1/h]}$ | [48] |
| $s_1$ | Positive parameters | $1 \times 10^3 \text{ [cm}^3/\text{g]}$ | [48] |
| $s_2$ | Positive parameters | $1 \times 10^3 \text{ [cm}^3/\text{g]}$ | [48] |

*: linear increase from minimum to maximum value depending on oxygen levels

**: linear decrease from maximum to minimum value depending on oxygen levels

***: initial value in the absence of drug.

****: initial value in normoxia conditions.
**Supplementary Table S2.** Value of parameter $k_1$ used for fitting the model to the two experimental studies. The parameter $k_1$ describes the dependence of cancer cell proliferation on the local oxygen concentration (Eq. 18).

| Experimental study     | $k_1$     |
|------------------------|-----------|
| Pham et al. [73]       | 0.47 day$^{-1}$ |
| Conley et al. [74]     | 0.77 day$^{-1}$ |
Supplementary Figure S1. Representative time intensity curves for (A) Control group and (B) Doxil (2 mg/kg) group on Day18 (end of 1st treatment cycle) and definition of parameters measured: PI (peak intensity), AUC (area under the curve), rise time and mean transit time. According to previous studies [75, 76], i) peak intensity and area under the curve are related to blood volume and ii) rise time and mean transit time are related to blood flow/perfusion. Black dots are the raw data and the red line is the fit to the data for the calculation of the parameters measured.
Supplementary Figure S2. Low and more frequent doses of Doxil (1 mg/kg and 2 mg/kg) combined with aPDL1 treatment result in a higher peak intensity of contrast enhanced ultrasound agents (i.e. microbubbles) -measured with ultrasound- compared with control, aPDL1 alone and group with high but less frequent doses of Doxil 6 mg/kg combined with ICB aPDL1. No effect is observed in the total integrated contrast enhancement, measured by the area under the time-intensity curve (AUC). Statistical analyses were performed by comparing the Doxil 1 mg/kg and Doxil 2 mg/kg with all other treatment groups *, p ≤ 0.05, determined by t-test. Data presented as mean ± SEM (n = 6 mice per group).
Supplementary Figure S3. Low and more frequent doses of the nanomedicine Doxil normalize the tumor mechanical microenvironment in MCA205 tumors. (A) Quantification of the average elastic modulus of the tumors on Days 11, 18 and 25 using SWE. (B) Mean transit time, (C) rise time, (D) peak intensity and (E) area under the time-intensity curve (AUC) of contrast agents for the different treatment groups using DCEUS. (F) Interstitial fluid pressure (IFP) levels at the end of the treatment protocol. Statistical analyses were performed by comparing the Doxil 6 mg/kg group with the control and aPDL1 groups * and the Doxil 1 mg/kg and Doxil 2 mg/kg with all other treatment groups **, p ≤ 0.05, determined by t-test. Data presented as mean ± SEM (n = 6 mice per group).
Supplementary Figure S4. (A) Representative image showing the opening of the tumor after a tumor opening experiment as a measure of growth-induced, residual stress. After tumor excision, a cut was made ~80% of the thickness of tumor along the main axis and the tumor opens up as a result of stress release and tissue relaxation. Measurements of tumor opening in (B) 4T1 and (C) MCA205 tumors, indicating that treatment with low and more frequent doses of Doxil (1 mg/kg and 2 mg/kg) combined with aPDL1 treatment leads to lower values of tumor relaxation compared to control, aPDL1 alone and the 6 mg/kg Doxil group, resulting in lower levels of growth-induced stress. Furthermore, the tumor opening decreases significantly in the 6 mg/kg Doxil group compared to control and aPDL1 monotherapy groups. Statistical analyses were performed by comparing the Doxil 6 mg/kg group with the control or aPDL1 monotherapy groups * and the Doxil 1 mg/kg and Doxil 2 mg/kg with all other treatment groups **, p ≤ 0.05, determined by t-test. Data presented as mean ± SEM (n = 6 mice per group).
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