Three-Compartment Model of CAR T-cell Immunotherapy

Brendon de Jesus Rodrigues · Luciana R. Carvalho Barros · Regina C. Almeida

Abstract

Immunotherapy has gained great momentum with CAR T (chimeric antigen receptor) cellular therapy, in which the patient's T lymphocytes are genetically manipulated to recognize tumor-specific antigens to increase elimination efficiency. Although recently approved by FDA to treat B cell malignancies, issues such as dose, administration protocol, toxicity, resistance to immunotherapy, among others, remain open and are the subject of intense research nowadays. Improved CAR T cell immunotherapy requires a better understanding of the interplay between CAR T cell doses and tumor burden. We developed a three-compartment mathematical model to describe tumor response to CAR T cell immunotherapy in immunodeficient mouse models (NSG and SCID/beige) based on two published articles from literature. We modeled different receptors as CART 19BBz or CART 123, and different tumor targets as HDML-2 and RAJI. We considered interactions between tumor cells, effector T cells, and T cell differentiation into memory T cells; tumor-induced immunosuppressive effects, conversion of memory T cells into effector T cells in the presence of tumor cells, and individual specificities considered as uncertainties in the parameters of the model. The model was able to represent the two considered immunotherapy scenarios. For the HDML-2 scenario, the tumor is eliminated after the immunotherapy with the CAR T cells even in case of a challenge due to the memory T cells long-term immune protection. For the Raji tumor, expressing indoleamine 2,3-dioxygenase (IDO) activity, the model represented tumor dynamics even when IDO inhibitors are introduced. Using in silico studies considering different dosing quantities and tumor burden, we showed that the proposed model can represent the three possible outcomes: tumor elimination, equilibrium, and escape. We found that therapy effectiveness may also depend on small variations in the parameters' values, regarded as intrinsic individual specificities, as T cell proliferation capacity, as well as immunosuppressive tumor microenvironment factors. These issues may significantly reduce the chance of tumor elimination. In this way, the developed model provides potential use for assessing different CAR T cell protocols and associated efficacy without further in vivo experiments.

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1 Introduction

Adoptive cell therapies have been considered a major advance in the fight against cancer, especially those associated with the hematopoietic system [36]. In particular, therapy with CAR T cells has been successful in eliminating or relieving endurable types of lymphomas and leukemia. In 2017, the US Federal Drug Administration (FDA) approved the commercialization of two therapies with CAR T cells for the treatment of B cells hematopoietic system malignancies [24]. By the end of 2016, four different immune checkpoint blockade drugs were also approved for the treatment of lymphoma, melanoma, among others. Current and future advances in the engineering of CAR and new immunologic checkpoint inhibitor drugs offer promising perspectives in the treatment of cancer [24,55]. Notably, many challenges must be addressed as minimum effective T cell dose, subtypes of CAR T cells selection, adverse effects, combination with other types of therapy, and patient specificity.

CAR T cell immunotherapy is an adoptive cellular therapy in which T lymphocytes are taken from the blood of a patient, genetically modified to recognize antigens expressed by patient's tumor, submitted to *in vitro* expansion and reinjected into the patient. Insertion of CAR gene into T lymphocytes bestows the ability to recognize and directly attack tumor cells independently of HLA presentation [44]. One advantage of this type of immunotherapy comes from the generation of T cell memory persistence. The quantification and complete understanding of the process of how tumors generate and maintain T cell memory pool is still a recent research topic.

Tumor evasion of the immune system, a hallmark of cancer [26], have some mechanisms elucidated, such as the PD1/PD-L1 immune checkpoint [28]. The PD-1 (programmed death receptor), expressed on the surface of effector T cells causes a decrease in proliferation and also a reduction in the production of cytokines by these cells [18]. Several tumors express PD-L1 leading to tumor evasion [7]. Many others immune checkpoint molecules were described such as IDO, LAG3, and VISTA with high potential to be used as target therapy [2, 9]. IDO (Indoleamine 2,3dioxygenase) is an intracellular enzyme that has an inhibitory activity on T cells, and is overexpressed in several human cancers, including prostate, pancreas, breast, brain, and hematologic malignancies [6,
52].

Recent experimental studies have investigated the relationship between immunotherapy with CAR T cells and the development of immunological memory in cancer [42,11]. Ruella *et al* [42] evaluated the immunotherapy with CAR T 123 cells against a Hodgkin's Lymphoma and the response to a challenge in immunodeficient NSG mice. Immunocheckpoint blockade associated with CAR T cell therapy is also under investigation in models where CAR T cell therapy fail. In the latter case, CAR T 19 therapy against Raji lymphoma cell line was combined with IDO inhibitor (1-MT) [40]. Ninomyia *et al* [40] showed that tumor IDO activity can indeed inhibit CAR T 19 therapy, and the administration of an IDO inhibitor (1-MT) can restores IDO-positive tumor control. In the present work, we use data from both [42] and [40].

Mathematical models have contributed to the understanding of the mechanisms involved in immunotherapy [11,4,32,15,33], confronting hypotheses and testing different settings. Some models are used to evaluate the role of immunotherapy in tumor growth [47,30], while others aim to analyze the combination of therapies to potentiate response [14]. In this work we aim to model mathematically the relationship between immunotherapy with CAR T cells and the long-term immunological memory. Some mathematical models have been recently developed representing tumor immune response to CAR T immunotherapy, either by assessing toxicity, interaction with other cells of the immune system, healthy cells, and tumor cells[1,49,38]. Just a few, however, have discussed the immunological memory in cancer [27,23].

The mathematical model developed here is built based on the data presented in [42] and in [40], encompassing different receptors (CART 19BBz or CART 123), and also different tumor targets as (HDML-2 or RAJI). It includes the dynamics of the tumor, CAR T effector and memory T cells, allowing a broader model. We estimated the model parameters with the data presented in [42], and model simulations were able to describe the development of the tumor after immunotherapy with CAR T cells and after the challenge of reinjection of cancerous cells. Likewise, model calibration for the Raji-IDO

scenario allowed to investigate the role of the immunosuppressive model term. We also used the model as an in silico tool for a more comprehensive analysis to investigate different CAR T response rates depending on the relationship between the tumor burden and CAR T cell number; the therapy effectiveness due to inhibition by immunosuppressive tumor microenvironments (such as PD1 and IDO), and intrinsic individual specificities represented by the uncertainties in the parameters of the model. Since the number of CAR T cells is limited, the identification of effective CAR T cell dosing play a key role on clinical practice. The quantification of uncertainties in the modeling process to identify how they impact the system response is a fundamental step for the reliability of the desired predictions. Typically, without this quantification, computational models are of little applicability in the clinical setting. In this work we perform the analysis of the model taking into account uncertainties present in the values of the parameters. In a real case, such uncertainties may be associated with donor/tumor intrinsic characteristics. We used a simple sensitivity analysis technique, which allowed us to identify that the success of immunotherapy is closely associated with the tumor growth rate, CAR T cell inhibition, and mostly CAR T cell proliferation.

2 Materials and methods

2.1 Quantifying the tumor burden

In both [42] and [40], tumor burden in mice untreated and treated with CAR T is assessed by serial bioluminescence imaging. In [42], 2×10^6 cells of Hodgkin lymphoma with luciferase (HDML-2-LUC) were injected into NSG mice. After 42 days, mice were randomly assigned to receive no therapy (control), receive CAR T 123 immunotherapy, or immunotherapy with untranslated lymphocytes (UTD - untransduced T cells). In mice subjected to CAR immunotherapy, 2×10^6 CAR T 123 cells were injected at day 42, and the tumor growth is monitored by serial bioluminescence imaging for 250 days. The tumor grows approximately exponentially in untreated mice, and a rapid and permanent eradication of disease is observed in mice receiving CAR T 123 immunotherapy (Figure 4 of [42]). The establishment of the long-term immunological memory is verified by challenging the pre-treated mice at the time of 250 days with the injection of 1×10^6 cells of HDLM-2-LUC. The same number of cells was injected into untreated mice to form a new control group. Figure 5 of [42] showed tumor growth in mice belonging to the control group, while the mice previously treated with CAR T cells had no tumor growth due to the long-lasting protection afforded by immune memory.

In [40], the action of CAR T 19 cell immunotherapy against Raji tumor ($CD19^+$ lymphoma) that express or not the IDO enzyme in SCID/Beige mice was evaluated. This tumor is quite aggressive and develops rapidly. Seven days after the injection of 3×10^6 tumor cells, mice received 1×10^7 cells of CAR T 19 (CD19-CAR T). Tumor growth was monitored by bioluminescence imaging at 4- and 7-days post therapy. Tumor growth in mice of the Raji-IDO group was much higher than that of the Raji-Control group, demonstrating the effect of IDO on inhibiting therapy in the presence of CAR T 19 cells (Figure 2 of [40]). To investigate the effect of an IDO inhibitor (1-MT), mice injected with Raji-IDO were treated with an oral solution of 1-MT (5mg/mL) one day before receiving CAR T cells. Both 1-MT and CAR T therapies slow down tumor growth and this effect is intensified when these therapies were combined.

2.2 Model development

In this work, we focus on the development of a three-compartment mathematical model, using ordinary differential equations, in which we investigate the interactions between populations of tumor, effector T and memory T cells. As we are dealing with immunodeficient mice, we consider that the effector T cells come only from immunotherapy, represented by populations of CAR T lymphocytes (activated) that we denote by C_T. The population of memory T lymphocytes is denoted by C_M and that of tumor cells by T. We construct the model based on the following assumptions:

A1) A given dose of CAR T cells (a mixture of $CD8^+$ and $CD4^+$ cells) is introduced into the system. They are considered activated T cells and have a cytotoxic effect on tumor cells. Manufactured CAR T cells have antitumor activity and derive from a given $CD4^+$ and $CD8^+$ T cell subsets that receive a specific chimeric antigen receptor (CAR) expressed by the tumor [46,54]. CAR T 19 therapies are already approved for clinical use in patients bearing B-cell leukemias [35]. Other target proteins have been studied recently, such as CD20, CD22 and CD123, for example. CD123 is also expressed in many hematological malignancies, including acute myeloid leukemia, Hodgkin's lymphoma, acute lymphoblastic leukemia, among others, which makes it a potential antineoplastic target [12].

A2) CAR T cells have a death rate. By definition, effector T cells are activated cells that modulate both the immune response and inflammatory signaling. When they are no longer needed, they must die to avoid a persistent immune response, which could result in host damage, as in autoimmune diseases. For immunotherapy in immunodeficient mice presented in [42,40], it is shown that CAR T cells are not detected on blood examination after tumor elimination.

A3) Memory T cells are activated by tumor cells. This is a simplification of the intricate mechanism by which the tumor antigens activate the memory T cells. Here we consider only the effects associated with the activation of memory T cells by the tumor, which provides a rapid response to the presence of the target antigen presented by the tumor [41].

A4) Memory T cells have a death rate much smaller than that of CAR T cells. According to [41], once formed, a memory T cell has half-life of approximately 8-15 years. Thus, each memory can survive for decades, providing long-term protection.

A5) In the absence of immunosurveillance, the tumor growth is limited only by the available resources. Current literature presents many mathematical models to describe the growth of tumors; see, e.g., [39]. Here we use the classic model defined by a logistic law that states that the tumor initially grows rapidly but growth slows as the tumor increases [16].

A6) Upon contact, CAR T cells kill tumor cells at a constant rate. CAR enables T cells to bind tumor surface antigens, without the dependence on antigens expressed by tumors through their major histocompatibility complex (MHC). T cells release perforin and granzymes leading to tumor lysing. One CAR T cell is capable of killing dozens of tumor cells [8,31,21,45,25,5].

A7) CAR T cells are inhibited by tumor cells. This assumption describes the ability of cancer cells to evade the immune activity, a hallmark of the cancer [26]. It may represent, for example, the interaction of the ligand PDL1 expressed by cancer cells with the PD-1 receptor present on the surface of T cells. This binding inhibits the activation of new cytotoxic T cells in the lymph nodes and the subsequent recruitment for the tumor. Likewise, other immunosuppressive mechanisms (IDO, regulatory immune cells, etc.) [3], that ultimately induce T cell apoptosis, can be encompassed by this assumption.

A8) CAR T cells are converted into memory T cells at a certain constant rate. A portion of CAR T cells persists as memory T cells that keep antitumor antigen specificity and patient characteristic [29,43]. They have a lower activation threshold, which eases the secondary response to a future tumor recurrence [50].

A9) CAR T cells proliferate at a certain constant rate. The proliferation of the CAR T cells is a rather intricate mechanism, associated with the tumor microenvironment conditions [37]. We assume here that cells have a spontaneous growth rate, and that part of the population will eventually undergo phenotypic differentiation.

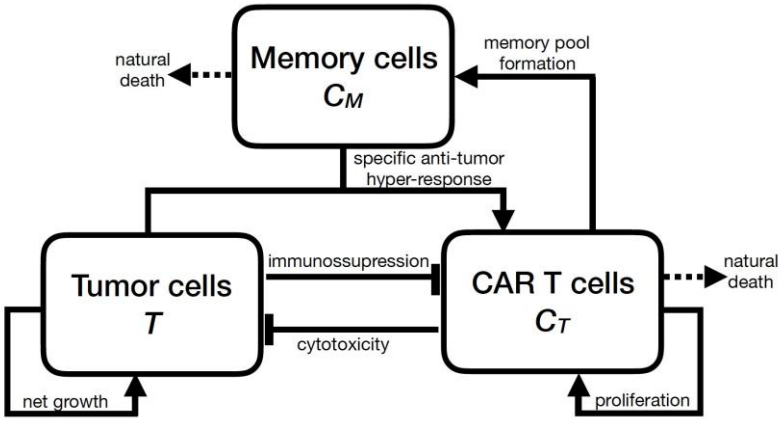


Fig. 1 Schematic description of the model structure. CAR T cells proliferate, have a cytotoxic effect on tumor cells, differentiate into memory cell, and die naturally or due to immunosuppressive mechanisms. Memory T cells are readily responsive to the tumor associated antigen so that, when they interact with tumor cells, they differentiate into effector T cells, producing a rapid response of the immune system.

The model was built by considering the key assumptions described above, and its structure is schematically described in Figure 1. The change of each cell density in time depends on the balance among all factors contributing for its increase and decrease. Each mechanism is modeled individually, which results in the following system of ordinary differential equations:

$$\frac{dC_T}{dt} = \phi C_T - \rho C_T - \mu_T C_T + \theta T C_M - \alpha T C_T \quad (1)$$

$$\frac{dC_M}{dt} = \epsilon \rho C_T - \theta T C_M - \mu_M C_M \quad (2)$$

$$\frac{dT}{dt} = rT(1 - bT) - \gamma T C_T \quad (3)$$

The meaning of the parameters (strictly positive real numbers) is given in the Table 1. Equation (1) models the dynamics of CAR T cells. They undergo an expansion due to proliferation at a rate φ, and have half-life $1/\mu_T$. According to the linear progression model described in [11,20,51], they quickly differentiate at a rate ρ into long-term memory T cells, which are assumed to provide long lasting protection to the specific tumor/antigen. This means that at any future time in which memory cells come into contact with same tumor cells, memory T cells are able to rapidly be converted into effectors T cells, readily activated to prevent tumor progression. Such mechanism is modeled by the term $\theta T C_M$. Finally, CAR T cells may be inhibited due to tumor modulated immunosuppressive mechanisms according to the term $\alpha T C_T$.

Table 1 Summary of the model parameters.

Parameter	Meaning	Unit
φ	C_T proliferation rate	day^{-1}
ρ	differentiation rate of C_T into C_M	day^{-1}
μ_T	C_T death rate	day^{-1}
θ	conversion coefficient of C_M into C_T due to interaction with T	$(cell \cdot day)^{-1}$
α	C_T inhibition coefficient due to interation with T	$(cell \cdot day)^{-1}$
	umerical response of the conversion of C_T into C_M	-
μ_M	C_M death rate	day^{-1}
r	maximum growth rate of T	day^{-1}
b	inverse of the tumor carrying capacity	$cell^{-1}$
γ	death coefficient induced by C_T	$(cell \cdot day)^{-1}$

Memory T cells C_M form the immunological memory, a key dynamic of the adaptive immune system [11,51]. Since we are dealing with immunodeficient mice, they are formed exclusively from differentiation of CAR T cells at a rate ρ. As mentioned, when in future contact with the same antigen bearing cancer cells, they immediately return to the effector phenotype at a per capita rate proportional to the tumor burden. In general, memory T cells have longevity, and therefore have a much lower mortality rate than the effector T cells [51], i.e., $\mu_M << \mu_T$. This dynamic is represented by the equation (2).

The response of tumor cells to the CAR T immunotherapy is modeled by the equation (3). We assume that, without immunotherapy, cancer cells grow subject to the limitation of available resources in the tumor microenvironment. This implies in representing tumor growth using a logistic model in which r is the maximum growth rate and $1/b$ is the maximum cell density that the available resources are capable of sustaining. CAR T cell immunotherapy acts by inhibiting tumor growth by cytotoxic action, causing a per capita mortality rate γC_T that depends on the number of effector T cells.

The model (1)-(3), representing the given set of assumptions, has ten parameters. Their estimation ultimately defines the desired immunotherapy scenario, to which the model parameters are estimated using data from [42] or [40]. Details are shown in the supplementary material. Mathematical equations were solved numerically using the explicit fourth order Runge-Kutta method [17]. We believe that the overall approach provides a framework for investigating the roles of CAR T dose, immunosuppressive tumor microenvironment and individual uncertainties on the therapy response. These issues are investigated in the next section.

3 Results: *In silico* experiments

We want to represent the setting in which immunodeficient mice are injected with tumor cells and, after some days, they are submitted to a CAR T cell immunotherapy. To the mathematical perspective, this requires defining initial conditions for the cell populations, amounting to set $T(0)$ as the injected tumor cells, and $C_T(0) = C_M(0) = 0$ cells. At the time of the immunotherapy, when tumor burden has already undergone significant growth, C_T receives the amount of CAR T cells. The cell populations are followed up to investigate tumor response and immunological memory formation. The procedure used for parameter inference is detailed in the supplemental material.

3.1 Model fits: CAR T 123 therapy eliminates HDLM-2 tumor, providing long-term protection; the immunotherapy with CAR T 19 on Raji tumor slows down its growth

Model parameters are estimated based on the data published in [42] and [40] for the CAR T 123 and CART 19 scenarios, respectively (see the supplementary material). The scenario described in [42] considers an initial condition of $T(0) = 2 \times 10^6$ HDLM-2 cells. Model simulation shows that the tumor grows apparently exponentially until it reaches about 2×10^7 cells in $t = 42$ days (Figure 2(a)). At this time, immunotherapy with CAR T 123 is performed, so that we impose $C_T = 2 \times 10^6$ cells at $t = 42$ days. Immunotherapy rapidly eliminates the population of tumor cells in a few hours. As C_T cells decay, phenotypic differentiation occurs giving rise to memory T cells C_M. Tumor cells and CAR T cell populations remain undetectable until $t = 250$ days. We observe the presence of long-term memory T cells, which slightly decline in time due to small mortality rate μ_M. The challenge is carried out at $t = 250$ days, when tumor cell population is set equal to 1×10^6 cells. The presence of tumor cells yields the conversion of C_M into C_T which is rapidly able to eliminate the new tumor. Afterwards, C_T undergoes rapid decay while part of memory T cells population is recovered. Tumor clearance remains until the end of simulation at day 500.

We next fit the model to a different scenario, described in [40], regarding a more aggressive Raji tumor. Beginning with $T(0) = 3{\times}10^6$ cells, the tumor reaches almost 1×10^8 cells at day 7, when $C_T = 1 \times 10^7$ cells of CAR T 19 cells is introduced. Raji-control tumors are inhibited by the immunotherapy, although not eliminated, and tumor cell population reaches 6×10^8 cells at day 14 (Figure 2(b)). Effector T cells undergo an expansion of 30% at day 9, from which decreases to extinction. The immunotherapy dose was not enough to lead to the formation of memory T cells.

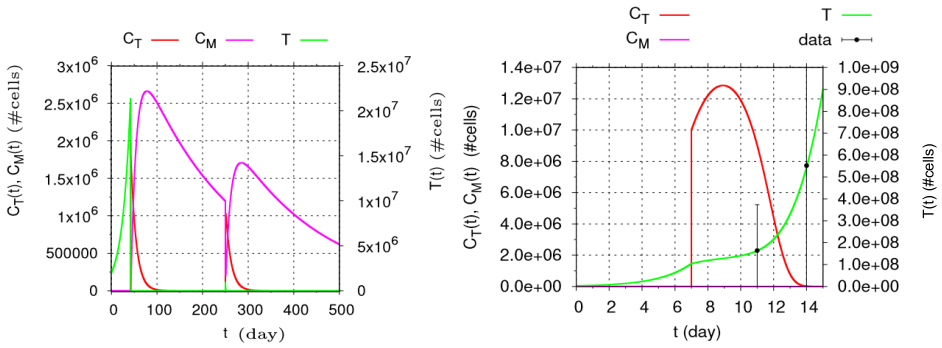


Fig. 2 Dynamics of T, C_T and C_M cell populations. (a) The immunotherapy with CAR T 123 on HDML-2 and challenge are performed at $t = 42$ and $t = 250$ days. Tumor is rapidly eliminated after C_T is introduced in the system. Soon after there is a decay of C_T, which is partialy converted into C_M. Tumor remains undetectable until day 250. Simulation is continued by carrying out a challenge at day 250. Upon contact with new tumor cells, C_M is converted into C_T, which rapidly eliminates the tumor. Afterwards, immunological memory is partialy recovered. (b) The immunotherapy with CAR T 19 on Raji-control is performed at day 7. There is an expansion of effector T cells, which can reduce growth but not eliminate the tumor. Effector T cells are practically extinct at the end of the simulation. There is no formation of memory cells. (Data extracted from [40].)

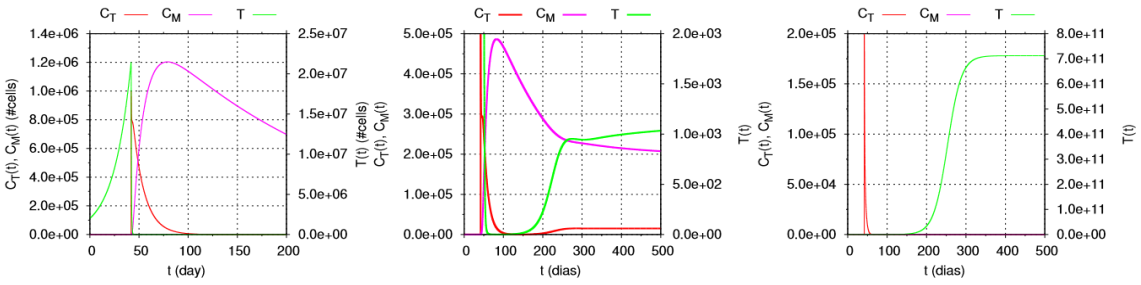
3.2 Insights on CAR T 123 dosing strategy into the elimination of HDLM-2 tumors

To first assess how the dose interferes with the response to the CAR T 123 immunotherapy, we perform three different simulations with therapy doses of 1×10^6, 5×10^5 and 2×10^5 *cells*. We use the same scenario described in Figure 2(a) and same model parameters, keeping the initial tumor burden of $T(0) = 2 \times 10^6$ cells. The resulting dynamics are shown in Figure 3(a)(c). A CAR T dose of 1×10^6 *cells* is able to perform tumor elimination, although the level of memory cells at $t = 200$ *days* is smaller than in the

case presented in Figure 2(a). Higher number of CAR T cells generates greater immunological memory pool. On the other hand, reducing the dose of CAR T cell to 5×10^5 *cells*, the tumor is not eliminated. It undergoes an intense decrease but resumes growth at day 150, eventually reaching a state in which it does not grow or shrink significantly, wherein the tumor is reduced to a very small (but not zero) value (Figure 3(b)). In this equilibrium state, both C_T and C_M s are non-zero, and therefore there is coexistence of the three cells populations. This is a typical configuration of tumor dormancy. Finally, reducing even more the CAR T dose to 2×10^5 *cells*, the tumor escapes from the immunotherapy. The tumor is initially reduced by therapy (not visualized because of the scale) but resumes growth and reaches the carrying capacity at around day 300 (Figure 3(c)). There is a complete and rapid extinction of the CAR T population and no formation of memory T cells.

Although not shown, it is worth remarking that those three tumor responses of elimination, equilibrium and escape can be reached by fixing the CAR T dose and increasing the tumor burden.

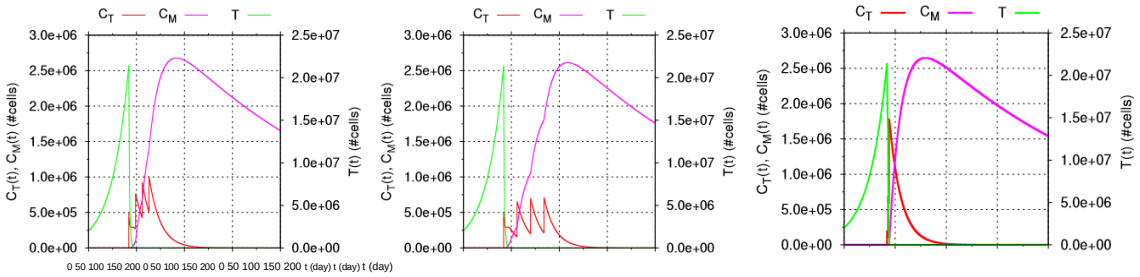
The next experiment explores the alternative possibility of CAR T cell dose fractionation. We select the same scenario described in Figure 2(a) with 1-time infusion of 2×10^6 *cells*, which promotes tumor elimination. Firstly, simulations were performed dividing the total dose into four equal fractions, infused at each seven or fourteen days. Figures 3(d) and 3(e) show that the dosing split does not interfere on the tumor elimination, which occurs in few days. Of note, a single dose of 5×10^5 CAR T cells is not able to eliminate the tumor burden, as shown in Figure 3(b). While in a single infusion case tumor decreases but resumes growth until reaching an equilibrium, the used fractionated infusions prevent tumor regrowth. As well as in Figure 2(a), immunological memory is formed, and the peak of memory cells is like that of single total dose infusion, although a certain delay is observed due to dose fractionation. Such delay ultimately yields a greater formation of immunological memory at day 200. Specifically, the number of memory cells at that time is 10% and 17% for 7- and 14-days rest time between doses, respectively. Although this feature could be seen as an advantage towards fractionated infusions, long rest periods between doses cannot be used because CAR T cells do not survive in culture medium for such long time. Alternatively, a simulation was performed for a more realistic fractionated immunotherapy, as investigated in [19]. In that work, patients with r/r CD19+ ALL were treated with three fractionated infusions over 3 days with increasing doses (10%: d1, 30%: d2, and 60%: d3). It was shown that such treatment protocol does not compromise effectiveness while reducing toxicity effects. Figure 3(f) shows the *in silico* predictions using this protocol. Like in a 1-time infusion protocol shown in Figure 2(a), tumor is rapidly eliminated, effector T cells vanish in 100 day while immunologic memory amounts for 1.5×10^6 cells at day 200.



(a) CAR T dose: 1×10^6 cells **Tumor elimination**

(b) CAR T dose: 5×10^5 cells **Tumor dormancy**

(c) CAR T dose with 2×10^5 cells **Tumor escape**



(d) 4 doses of 5×10^5 CAR T cells every 7 days

(e) 4 doses of 5×10^5 CAR T cells every 14 days

(f) 3 fractionated infusions over 3 days (10%:d1, 30%:d2, and 60%:d3)

Fig. 3 *In silico* prediction of the immunotherapy response to different CAR T cell dose. Initial HDLM-2 tumor burden amounts 2×10^6 *cells*. Top row: (a) With 10^6 CAR T cells injection, tumor elimination occurs around day 100; around 7×10^5 memory cells remain at t = 200 *days*; (b) Half of the previous CAR T cell dose (5×10^5) induces a strong decline in the tumor burden, together with a decrease in the number of CAR T cells. However, tumor rapidly resumes growth. After day 250, the three cell populations slowly change towards an equilibrium state, in which a small pool of tumor cells coexists with the CAR T and memory T cells populations; (c) 2×10^5 CAR T cells dose is not able to control the tumor, which escapes and reaches the carrying capacity at day 350. The fast decay of CAR T cells prevents the formation of a memory cell population. Bottom row: The total CAR T dose of 2×10^6 *cells* is fractionated into four equal portions and administered every (d) 7 days or (e) 14 days; (f) the dose if fractionated into 3 infusions, of increasing dose values, over 3 days. In all cases (d)-(f), the tumor is eliminated in a few days, followed by a decrease of the effector T cells. Fractionated infusions lead to the formation of memory T cells, although the quantity depends on the rest time between doses.

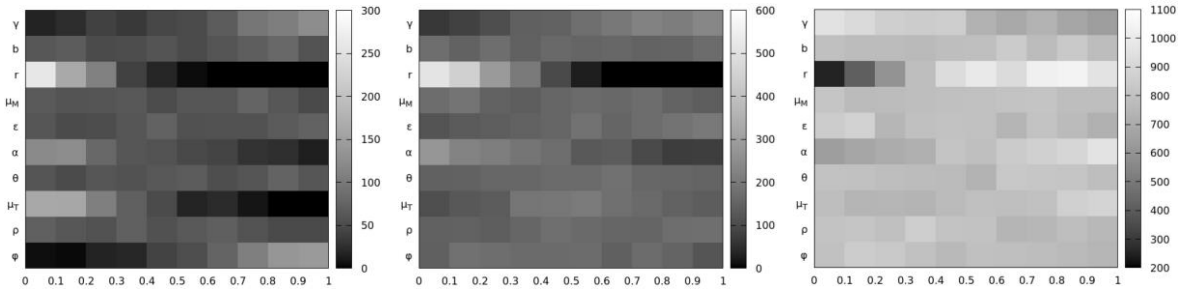
3.3 How do parameter uncertainties impact the elimination of HDLM-2 tumors?

We now use *in silico* experiments to investigate how parameter uncertainties impact the CAR T 123 immunotherapy outcomes. In the absence of information that characterizes the uncertainties in the parameters of the model, we assume that each parameter is a random variable with uniform distribution in the range limited by 20% of the reference values indicated in Supplementary Table 1. For the same initial conditions defined in Figure 2(a), we solve the model for 10,000 randomly selected samples of the parameter vector and determine the tumor response to the CAR T cells immunotherapy. We then build the three heatmaps shown in Figure 4, that display the frequency of occurrence of the elimination, equilibrium, and escape of the tumor. The simulations indicated that the uncertainties in the parameters can drastically reduce the chance therapy success: of the 10,000 cases considered, the therapy was successful in only 5% of them (507 cases). The frequency of the elimination is quite heterogeneous, being smaller for more aggressive (more proliferative) tumors (higher r) and higher for less aggressive tumors (smaller r), with a lower rate of CAR T cell death (smaller μ_T), less ability to evade the immune system (smaller α), and higher CAR T cell proliferation rate (higher φ). The equilibrium and escape responses, which occurred in 18% and 77% of cases, respectively, have more homogeneous occurrence frequencies than when the elimination occurs. This means that these responses are more likely to occur, with a decreasing dependence on the values of all parameters. Specifically, only the parameters r and α significantly interfered with the equilibrium response in 1853 cases. Notice that system equilibrium was more frequently in less aggressive tumors (smaller r) and lower effects of inhibition by tumor on CAR T cells (smaller α). Finally, tumor escape was the most prevalent response, occurring in 7640 cases. Except

for the tumor proliferation rate, none of the parameters play significant role on tumor escape. Overall, the performed simulations indicate that tumor response is very sensitive to parameter uncertainties, and tumor escape is most likely to occur. Among the parameters, those associated with the tumor proliferation (r), CAR T cell inhibition (α), and CAR T cell proliferation (φ) are the most influential for tumor elimination.

3.4 The effect of inhibitors of immunosuppressive tumor microenvironments

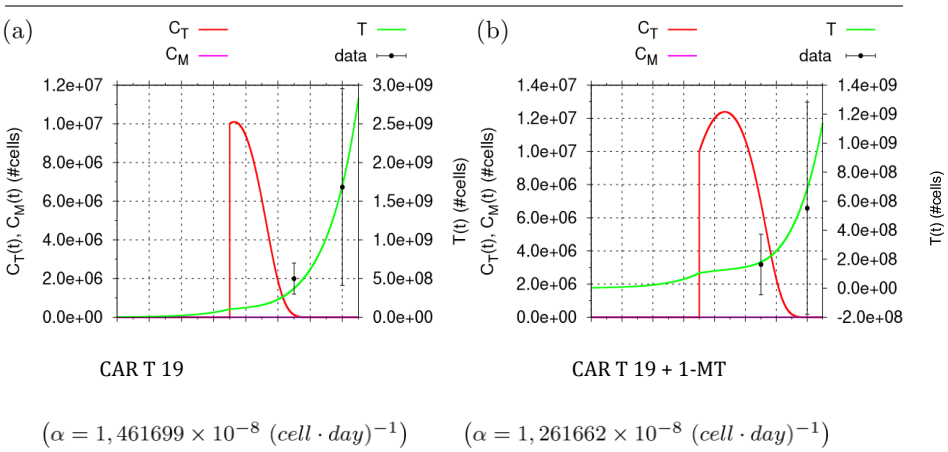
Our model includes the term αTC_T of equation (1) to describe tumor-modulated immunosuppressive mechanisms. Higher α value imply in stronger immunosuppressive mechanism. To check how this term allows investigating the blocking action of these mechanisms and, at the same time, how the model deals with different tumor and CAR T cell, we select data from [40] that presents the action of CAR T 19 cell immunotherapy against CD19 + lymphoma that express the indoleamine 2,3-dioxygenase (IDO) enzyme in mice. We estimated the parameters for immunotherapy with CAR T 19 cells alone or combined



(a) Elimination (5%) (b) Equilibrium (18%) (c) Escape (77%)

Fig. 4 Frequency of occurrence of elimination (a), equilibrium (b), and escape (c) of the tumor for the scenario in which the initial HDLM-2 tumor burden is 2×10^6 *cells* and the immunotherapy with 2×10^6 *cells* CAR T is performed at day 7. Darker colors indicate lower frequency. All parameters are assumed to be uncertain, being uniform distributed random variables with range limited by 20% of the reference values indicated in Supplementary Table 1. Horizontal axes are associated with the normalized parameter values. We evaluated 10,000 cases by randomly sampling the parameter space. Tumor escape is more likely to occur, followed by the equilibrium and elimination. The respective percentage of these responses are indicated in parentheses.

with 1-MT (1-methyl-tryptophan), an IDO inhibitor. The details associated with the calibration of the parameters are presented in the supplementary material. Figure 5 shows system responses for these two scenarios, obtained using the same parameter values, except that of the parameter α. The calibration procedure performed using the available experimental data published in [40] yielded the values indicated in Figure 5. The smaller α value obtained when 1MT was used allowed a greater expansion of CAR T cells after infusion which in turn provided a stronger control on the tumor growth than that promoted by the CAR T cells without 1-MT. Of note, in both cases the CAR T 19 dose was not able to eliminate the tumor, which eventually escapes, nor to give rise to memory cells. These simulations show that the model could adapt to the present scenario of a more aggressive tumor than that in [42], with a tumor evasion mechanism. Moreover, the model could capture the effect of an inhibitor of the evasion mechanism by representing well the decrease of the tumor growth.



CAR T 19 CAR T 19 + 1-MT

$(\alpha = 1,461699 \times 10^{-8}\ (cell \cdot day)^{-1})$ $(\alpha = 1,261662 \times 10^{-8}\ (cell \cdot day)^{-1})$

Fig. 5 System response to: (a) CAR T 19 immunotherapy; (b) CAR T 19 with IDO inhibitor (1-MT). Initial Raji-IDO tumor burden is 3×10^6 *cells*. At day 7, 1×10^7 CAR T 19 cells were introduced and were able to reduce the velocity of the tumor growth at some extent. Such reduction was more significant when CAR T 19 therapy was combined with the IDO inhibitor (1-MT): the number of tumor cells were less than half of that without 1-MT at day 15. Model parameter α was estimated for these two cases (values shown in parentheses), and was responsible to capture the effect of IDO inhibition and the 1-MT. Its value decreased for the latter case, being small enough to promote a higher expansion of the CAR T cells, and ultimately leading to a more effective control on the tumor growth. However, both therapies were not able either to eliminate the tumor or build memory cells. (Data extracted from [40].)

4 Discussion

CAR T cell therapies are spreading across hematological cancers and is already product of big pharma companies as Novartis and Gillead [34]. On the road, there are new CAR designs, including new antigen targets, different CAR affinity [22] and expansion protocols [13].

We develop a three-compartment mathematical model to describe tumor response to CAR T cell immunotherapy in immunodeficient mouse models (NSG and SCID/beige) based on two published articles from literature. In a general CAR T cell therapy model, independently of the recognized antigen, we modeled different receptors as CART 19BBz and CART 123, and different tumor targets as HDML-2 and RAJI. HDML-2 tumor model was used as a low proliferation, less aggressive tumor model where CAR T cell therapy is effective on tumor elimination. On the other hand, RAJI was chosen from its high proliferation and escape from CAR T cell therapy. On RAJI model we also include explicitly immune checkpoint inhibitor as IDO in order to estimate this component on CAR T/tumor cell interaction. Therefore, the model could adapt itself to different treatment and tumor scenarios. The adopted structure of the model allows identifying each individual mechanism in a more transparent way. Donor/tumor-microenvironment specificities are considered as uncertainties in the parameters of the model and estimated by model calibration. The model was able to represent tumor elimination after immunotherapy with CAR T 123 cells even in case of a new tumor challenge due to memory T cells long-term protection for HDML-2 target. The change of CAR T cells from effector to memory cells and their long-term persistency as CAR T memory cells were also demonstrated by [44] and our previous work with RS4;11 B-ALL model using 19BBz CAR T [10]. For the CAR T 19 therapy and RAJI target scenario, the model represented well the tumor dynamics with or without IDO inhibitor. We performed a few *in silico* studies to highlight how they might contribute to a better understanding of the underlying processes. We found that the determination of the dose of CAR T cell is a critical factor for the success of the immunotherapy. A previous model already considered CAR T cell proliferation in response to antigen burden, but memory CAR T were not considered, neither the effect of tumor inhibition of CAR T cells [53]. Another interesting mathematical model was made upon tisagenlecleucel-treated patient data [48]. This model was adapted from previous empirical model of immune response to bacterial/viral infections. They captured T cell expansion, contraction and persistence like our model does, including

CAR T memory population. Their model was calibrated on patients' data, and different from ours, no difference in dose response was detected. They attributed this result to CAR T cell proliferation capacity *in vivo*. We partially agree, but there is a possibility that obtained data from humans do not present very different CAR T cell dose (especially including only tisagenlecleucel clinical trials). Considering mouse model data, where CAR T cell dose varies by thousands, we do observe a dose effect, especially on aggressive, high proliferative tumors as NALM-6 cell line [13,10].

Another advantage of our model is the calculation of therapy effectiveness. Overall therapy effectiveness may depend on intrinsic individual specificities, regarded here as small variations in the model parameters' values. In the studied case, such parameter uncertainties drastically reduced the chance of tumor elimination to less than 10%. Additional *in silico* experiments can be conducted to identify, for example, the smallest dose to increase success chance in view of a setting with possible uncertainties. We have also shown that a fractionation of dose appears to be as effective as a single dose, and the rest periods between infusions might favor long-term immunological memory. These results corroborate with previous clinical trials using fractioned CAR T cell dose with similar effectiveness to single dose and persistence of CAR T cells on the blood 20 months after therapy [34].

We identified that uncertainties associated to the tumor proliferation and ability to inhibit the CAR T cells, and CAR T cell proliferation and death are the most significant to therapy success in eliminating the tumors. This opens room for investigating other chimeric antigen T-cell receptors with different target antigen affinities and the blockade of immune checkpoints to boost efficacy and safety. In our model, we did not consider CAR affinity for each antigen as an explicitly parameter, considering it as a result of tumor lysis by CAR T cells. Another aspect that we did not take on consideration is the toxicity effect of CAR T cell therapy (cytokine release syndrome - CRS), because our model is based on immunodeficient mouse model that lacks this effect. For human data, Hanson et al. [27] made a mathematical model to CAR T cell therapy for B-ALL emphasizing cytokines and CRS, also considering CAR T effector and memory cells.

Overall, the developed mathematical model may help to shed lights on the structure of treatment protocol. To this end, model must be calibrated by using one *in vivo* experimental data describing the tumor growth without and with treatment, and *in vitro* lysing data. Once calibrated, the model allows exploring alternative ways of scheduling and infusion dose in view of the current setting specificities, including parameter uncertainties, to elicit the one with higher chance of success. The model provides an *in silico* tool for assessing different issues associated with the therapy such as how CAR T cell dosing can be adjusted according to tumor burden, CAR T cell infusion protocols, immunosuppressive mechanisms, among others, without further *in vivo* experiments.

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