CAR-T cell Goes on a Mathematical Model

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Abstract

CAR-T cell immunotherapy is a great advance in hematological cancers treatment. New CARs and therapy schemes are being developed and a mathematical model could contribute to a rational design of treatment. Here we comment and show new results with previously published models of CAR-T cell therapy, emphasizing the contribution of initial tumor load, the proliferation of CAR-T cell and inhibition of CAR-T cell activity by the tumor resulting in different scenarios as tumor escape, equilibrium (stable disease) and tumor elimination.

Keywords: CAR-T cell, Immunotherapy, Mathematical model

Introduction

Hundreds of new clinical trials were recently launched using chimeric antigen receptor-bearing T cells (CAR-T cell). Concentrated on hematological malignancies, with a 90% overall survival rate for 2 months on acute lymphoid leukemia (ALL-B) in young patients after two or more lines of therapy [1]. The most surprising results were the long-lasting effect of this therapy, resulting in more than 50% overall survival after a year follow-up [2]. For several other hematological cancers, there is a significant improvement in treatment in the last few years [3]. This has pushed developments on new CAR designs to expand treatment to diseases, including solid cancers [4], as well as on new strategies for managing dose administration protocols [5] and toxicity to improve therapy outcomes [6].

Pre-clinical Mice Model and its Limitations

Since before this hype of clinical trials, pre-clinical models have been extensively used, especially immunodeficient mice as SCID-beige and NSG (NOD SCID gamma mouse). NSG mice are preferable because it lacks mature T and B lymphocytes as well as NK and functional macrophages and dendritic cells, making these animals suitable host for xenograft experiment as human tumors and CAR-T cells. Unfortunately, this model has several limitations, a lack of cytokine release syndrome (CRS) [7] and neurotoxic effects [8] being the most prevalent side-effects of CAR-T cell therapy. These side-effects are caused, at least in part, by an increase in mouse IL-6, IL-1 and nitric oxide (NO) produced by macrophages [9]. Several new targets and tumor models are being tested on these animals which serve as a proof-of-concept for new clinical trials but fail to predict off-targets and side-effects. Off-targets are also of great concern on new targets CAR design and they cannot be predicted based on animal model studies, because of inter-species differences.

CAR-T cell expansion in vivo is one of the main points of CAR-T cell therapy, but it is still unknown why some patients have a great expansion while others fail. Cycles of previous treatments are pointed as main factors because it affects T cells and its progenitors [10]. On the other hand, the mice model also lacks this parameter because healthy donors are the source of T cells for these experiments. A mathematical model based on mice experiments could represent and accept small differences in CAR-T cell proliferation from different healthy donors. However, the same model could fail to explain the great difference in CAR-T cell expansion in vivo when these cells are derived from patients, because of the major difference in cell proliferation. Afterall, CAR-T cell expansion is one of the main concerns and drawbacks of CAR-T cell
therapy [11,12]. In a mathematical model, we use in vitro expansion data as a proxy to infer the expansion of CAR-T cell expansion in vivo. We confirm that possibility by real measurements of luciferin luminescence and the model could be adjusted for each patient based on in vitro data.

Another possible deficiency in the mouse model is the lack of studies on tumor escape. Recently, clinical trials reported the loss of antigen as an important mechanism of CAR-T cell therapy failure in patients [11,13], but there is no tumor recurrence in the animal model because of antigen loss.

**Model-based Approach for the CAR-T-cell Immunotherapy**

Mathematical models already contributed to the understanding of immune system mechanisms such as macrophage function [14,15], memory formation to viral infection [15], and immunotherapies [16]. Other mathematical and computational models of immunotherapies were reviewed by Konstorum et al. [17]. The interest in modeling immunotherapies is to discover the most important factor impacting the results, in order to aid a rational therapy design and possibly to predict the effect of the therapy based on certain conditions. Although we can expect great contributions of mathematical models, they are not used in the clinics until now, possibly because individual patient data are not shared and accurate models cannot be constructed based on population data.

Lack of immune system and non-solid tumor xenografts simplifies experiments read-outs for CAR-T cell tests in a mathematical point-of-view. Recently, an effort to build mathematical models to explain CAR-T cell and tumor behavior have been proposed using both human and pre-clinical data [18,19]. Different from machine learning methods, mathematical models did not need thousands of experiments/measurements and are based on known mechanistic features.

In this communication, we discuss the construction of a three-compartment CAR-T-cell immunotherapy model to characterize the relationship between CAR-T cell therapy leukemia/lymphoma progression on an immunodeficient mouse model.

We consider three main compartments: effector CAR-T cells, memory CAR-T cells, and tumor cells. Although untransduced T cells are also injected on mice it is not considered on our model because of its potential and influence on CAR-T cell activity are not directly measurable. A schematic representation of the nonspatial interactions between tumor cells and CAR-T cells (memory and effector), and the corresponding general mathematical model (modified from [29]) is given in Figure 1.

Following the notation used in Eftimie et al. [20], the indicated functions have the following meaning:
- \( f(T) \) specifies the density dependence growth of tumor cells.
- \( p_{i,i} = C_{T}, C_{M} \) specifies the production of cells of type i.
- \( d_{i,i} = C_{T}, C_{M}, T \) specifies the inhibition of cells of type i.
- \( a_{i,i} = C_{T}, C_{M} \) specifies natural death (apoptosis/decay) of cells of type i.

Possible models can be built by defining different

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**Figure 1:** Tree-compartment mathematical model.

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functions that are modulated by parameters, which have to be estimated based on available data. Function \( f(T) \) (tumor growth) allows accounting cell density dependence on proliferation and apoptosis of tumor cells. Typical choices are exponential, logistic, and Gompertz functions (see [21] for further models). According to Johnson et al. [22], growth cell kinetics is better described considering an allee effect at low cell densities. Rodrigues et al. [29] used the logistic growth kinetic \( f(T) = r(1 - bT) \), implying the need of estimating the growth rate \( r \) and the carrying capacity \( (1/b) \) parameters. Such choice and estimation were defined in accordance with data published in Ruella et al. [23] that shows a saturation profile for untreated tumors. Of note, for aggressive tumors that do not reach such a saturation profile, exponential kinetics with a constant growth rate \( f(T) = r \) are more likely to represent the dynamics. In vivo readout used to extract values was the bioluminescent signal from tumor cells, acquired at several time points in a day/week scale. The readout is always total tumor cell bioluminescent signal, as a surrogate measure of tumor cell number [24] (more details below).

The induced tumor death treatment is modeled by function \( d^T(T, C^T) \). This function represents the effect of CAR-T cells cytotoxicity upon contact with tumor cells. It depends on CAR-T and tumor cell densities, and a killing rate parameter that may also depend on time to represent tumor resistance to the therapy. Such declining efficacy depends on CAR-T and tumor cell densities, and a killing rate parameter that may also depend on time to represent tumor resistance to the therapy. Such declining efficacy depends on time.

The remainder functions to be defined are \( p_{CT}(C_T) \), \( a_{CT}(C_T) \), and \( a_{CM}(C_M) \). The last two terms account for mortality of effector and memory T cells, and usually follow exponential decay laws which amount to set \( a_{CT}(C_T) = (\rho + \mu_T)C_T \), and \( a_{CM}(C_M) = \mu_M C_M \). It was notice that the effector T cell mortality rate includes both the rate associated with effector-to-memory differentiation and the natural mortality \( \mu_T \).

Since memory T cells have longevity and therefore have a much lower mortality rate than the effector T cells, their death rates satisfy the relationship \( \mu_T > > \mu_M \). Finally, the proliferation of effector CAR-T cells in vivo is modeled by the term \( P_{CT}(C_T) \). Since there is evidence that expansion in vivo depends on the tumor burden, this term could also depend on the number of tumor cells. Also remark that the overall CAR-T cell expansion in the model results from the balancing effect of proliferation, natural decay, and differentiation of the CAR-T cells, which implicitly takes into account the tumor burden. Based on this, we set \( P_{CT}(C_T) = \varphi C_T \) [29].

**CAR-T cells dynamics**

CAR-T cells production starts with the retrieval of peripheral blood mononuclear cells (PBMC) from healthy donors, in the case of animal experiments. Then, in vitro activation and expansion of CAR-T cells leads to CD45RO+CCR7- effector memory phenotype which we call effector CAR-T cell \( (C_T) \). CAR-T cell phenotype varies according to cell expansion conditions for example high IL-2, low IL-2 or IL-7/IL-15 cytokines [26-28]. Figures 2-4 represent several scenarios of CAR-T cell therapy and tumor, effector and memory CAR-T cells behavior.

**Model Limitations**

Obtaining numeric values as the number of tumor cells in vivo is a real challenge, as experiments do not quantify cell numbers directly. The most used technique...
Figure 2: Tumor, effector and memory CAR-T cells dynamics in several scenarios. Dynamics of tumor cells (left column), CAR-T cells (center column), and memory T cells (right column) for three different initial tumor burden: (a-c) $2 \times 10^6$ cells (top row), (d-f) $5 \times 10^6$ cells (center row), and (g-i) $1 \times 10^7$ cells (bottom row). Three initial CAR-T doses are considered in each plot: $2 \times 10^6$ cells (red), $5 \times 10^5$ cells (green), and $2 \times 10^5$ cells (blue).

Figure 3: High CAR-T cell inhibition from tumor leads to tumor escape and absence of CAR-T cell memory. For fixed initial tumor burden of $2 \times 10^6$ cells and CAR-T dose of $2 \times 10^6$ cells, the dynamics of tumor cells (left), CAR-T cells (center), and memory T cells (right) are shown for increasing values of the CAR-T cell inhibition parameter $\alpha$: for values below $2.02 \times 10^{-7}$ (cell · day)$^{-1}$, tumor elimination occurs (in red); for $2.03 \times 10^{-7}$ (cell · day)$^{-1} \leq \alpha \leq 3.47 \times 10^{-7}$ (cell · day)$^{-1}$, tumor reaches an equilibrium state (in green and blue, respectively); values above $3.48 \times 10^{-7}$ (cell · day)$^{-1}$ lead to tumor escape (in magenta).
that quantifies tumor and CAR-T cells number \textit{in vivo} is the measurement of photons captured by a CCD camera from luciferin luminescence (photons) in a certain period of time (usually seconds). Tumor cells are transfected/transduced stably with a luciferase sequence. Once animals receive luciferin, luciferase on tumor cells turn on the luminescence from luciferin and the instrument detects the photons/second. The technique also relies on the angle of the animal, the detector, and the concentration of luciferin on the body. Even if all measures are taken carefully, there is no direct correspondence of photons/time to the number of cells. However, the amount of luminescent measurement is directly proportional to the number of cells. As being so, we can estimate the number of cells based on the number of photons/time. Also, the technique has a low sensibility, turning the detection of a very small number of cells not possible. In that way, a very small number of tumors or CAR-T cells are not observed, introducing more uncertainties in the mathematical model. This very low number of cells that are not observable needs to be calibrated and the most probable value should be chose.

Although tumor kinetics \textit{in vivo} is represented by a logistic function, each tumor has it properties, including the great number of tumor cell death just after tumor inoculation into a mouse, especially on a xenotransplant situation. The remaining cells colonize the tumoral niche and the tumor grows until growth factors are consumed in hematological cancer, as leukemia/lymphoma. This massive death is not taken into account on a logistic function, but it fits the tumoral behavior.

Another important parameter that is not directly measured is the CAR-T cell capacity to kill a tumor cell within the animal. To circumvent this issue, we used data from a classical \textit{in vitro} cytotoxicity experiments where CAR-T cell and tumor cells are put together for 4 hours period of time and at different ratios. In the end, there is the percentage of tumor cell death and a dose-dependent manner that we use to calibrate the potential capacity of CAR-T cell to kill a tumor cell. This parameter is then tested on data obtained from \textit{in vivo} experiments.

\textbf{Perspectives}

Using data from the literature, we show how the model can be constructed, and the remaining challenges towards model validation. Through \textit{in silico} experiments, we show how individual specificities and immunosuppressive tumor microenvironments can impact treatment success, and the modeling resources to test possible strategies to circumvent them. Figures 2-4 show how different outcomes (tumor elimination, equilibrium or escape) are obtained depending on tumor burden, therapy dose, and individual specificities. Although the combination of mechanisms is what ultimately defines the outcome, \textit{in silico} experiments have shown that tumor aggressiveness, the dose of therapy, immunosuppressive effects of the tumor microenvironment, and the \textit{in vivo} CAR-T cells expansion capacity are the main factors that contribute to the success of immunotherapy. We remark that we do not model CAR-T cell constructions explicitly, but we are able to consider different CAR-T cell constructs by the parameter values extracted from \textit{in vitro} and \textit{in vivo} experiments. As different CARs confer different characteristics for T cells, these novel properties enter on the model as different values for the same parameter. For example, a higher and faster \textit{in vivo} proliferation of 1928ζ CAR-T cell compared to 19BBζ CAR enters as a higher proliferation rate value in the model. Also, the very long persistence of CAR 123 found \textit{in vivo} is applied as a very low death rate of CAR-T memory cells in this model. The developed model can be applied to reduce
the use of animals in research and also the time and cost of pre-clinical tests. For example, a CAR-T cell dosing or experiment could be avoided once the model is applied in a single dose experiment.

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