**Title**: Mathematical model suggests current CAR-macrophage dosage is efficient to low pre-infusion tumour burden but refractory to high tumour burden

**Author:** Shilian Xu1,2,\*, Maoxuan Liu3,\*

**Affiliation:** 1Department of Environment and Genetics, School of Agriculture, Biomedicine and Environment, La Trobe University, Bundoora, Australia,

2Department of Mathematics and Statistics, La Trobe University, Bundoora, Australia,

3Guangdong Immune Cell Therapy Engineering and Technology Research Center, Center for Protein and Cell-Based Drugs, Institute of Biomedicine and Biotechnology, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China

\*To whom correspondence may be addressed: [Shilian.Xu@latrobe.edu.au](mailto:Shilian.Xu@latrobe.edu.au), [mx.liu@siat.ac.cn](mailto:mx.liu@siat.ac.cn)

This pdf file includes:

**Supplementary text**

**Figures S1 to S3**

**Tables S1 to S6**

**Table of Contents**

1. Parameter Estimation
   1. Estimation of Raji Growth Kinetics in Absence of CAR-Macrophage or Normal Macrophage
   2. Estimation of CAR-Macrophage and Normal Macrophage Phagocytosing Parameters with Unsaturated, Semi-Saturated and Saturated Model
2. CAR-macrophage Kinetics with Raji Cells
3. **Parameter estimation**

This section first describes the estimation of Raji cell growth kinetics in the absence of CAR-macrophages or normal macrophages, utilizing the logistic growth model (System 1, Methods). Next, we describe the estimation of CAR-macrophage phagocytosing kinetics with unsaturated phagocytosing model (System 2, Methods), semi-saturated phagocytosing model with increase of Raji cell concentration (System 3, Methods), semi-saturated phagocytosing model with increase of CAR macrophage cell concentration (System 4, Methods), saturated phagocytosing model (System 5, Methods) and saturated phagocytosing model incorporating additional macrophage death due to phagocytosis (System 6, Methods).

* 1. Estimation of Raji Growth Kinetics in Absence of CAR-Macrophage or Normal Macrophage

In this section, we fitted the logistic growth model (System 1, Methods) to Raji proliferation assay data, as provided in Reference [1]. Our analysis yielded a per capita growth rate , a maximum Raji capacity and a sum of squared error . The fitted curve is shown in Fig. S1.

A graph of a number of cells

Description automatically generated

**Figure 1. Fitted curve of Raji proliferation assay data.** The figure displays the logistic model fit to the Raji proliferation assay data provided in Reference [1]. Red circles represent the experimental data points, while the blue line illustrates the estimated values and the fitted curve from the logistic model.

* 1. Estimation of CAR-Macrophage and Normal Macrophage Phagocytosing Parameters with Unsaturated, Semi-Saturated and Saturated Model

This section details the estimation of CAR-macrophage phagocytosing parameters. we will use the following model: unsaturated phagocytosing model (System 2, *Methods*), semi-saturated phagocytosing model with Raji cell concentration (System 3, *Methods*), semi-saturated phagocytosing model with CAR-macrophage concentration (System 4, *Methods*), saturated phagocytosing model (System 5, *Methods*) and saturated phagocytosing model with additional macrophage death due to phagocytosis (System 6, *Methods*). We used FACS-based phagocytosis assay data provided in Reference [2]. This data included three biological replicates, and we calculated their geometric mean to serve as a fourth biological replicate for our analysis.

Our initial findings show the fitted parameters, sum of squared errors (SSE), Akaike Information Criterion (AIC), and modified Akaike Information Criterion (AICc) for the unsaturated phagocytosing model (System 2, *Methods*). These are all detailed in Table S1.

**Table S1.** Estimated Parameters of CAR-Macrophage Phagocytosing Using the Unsaturated Phagocytosing Model (System 2, *Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Phagocytosing rate of CAR macrophage, ,  (mL/cells/day) | 1.2562 | 3.6161 | 1.1740 | 1.1344 |
| Sum of squared error (SSE) | 761.9056 | 749.8902 | 757.5007 | 794.2628 |
| Akaike information criterion (AIC) | 70.8793 | 70.6886 | 70.8097 | 71.3784 |
| modified Akaike information criterion (AICc) | 71.8793 | 71.6886 | 71.8097 | 72.3784 |

Fitted parameters, SSE, AIC and AICc of semi-saturated phagocytosing model (System 3, *Methods*) with increase of Raji cell concentration are given in Table S2.

**Table S2.** Estimated Parameters of CAR-Macrophage Phagocytosing Using the Semi-Saturated Model with Raji Cell Concentration (System 3, *Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Phagocytosing rate of CAR macrophage, ,  (mL/cells/day) | 4.8868 | 4.8868 | 5.0273 | 5.0747 |
| Saturation in phagocytosing efficiency as tumour cell concentration increases, ,  (mL/cells) | 9.9903 | 10.0812 | 10.0059 | 10.0709 |
| Sum of squared error (SSE) | 0.4891 | 0.4453 | 0.5551 | 0.4843 |
| Akaike information criterion (AIC) | -15.3328 | -16.4586 | -13.8138 | -15.4512 |
| modified Akaike information criterion (AICc) | -11.3328 | -12.4586 | -9.8138 | -11.4512 |

Fitted parameters, SSE, AIC and AICc of semi-saturated phagocytosing model with CAR-macrophage concentration (System 4, *Methods*) are given in Table S3.

**Table S3.** Estimated Parameters of CAR-Macrophage Phagocytosing Using the Semi-Saturated Model with CAR-Macrophage Concentration (System 4, *Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Phagocytosing rate of CAR macrophage, ,  (mL/cells/day) | 495.2441286 | 279.5276567 | 327.1255544 | 262.61057 |
| Saturation in phagocytosing efficiency as CAR-macrophage/ normal macrophage concentration increases, , (mL/cells) | 504.218887 | 344.4193475 | 356.3423361 | 291.08078 |
| Sum of squared error (SSE) | 0.3338 | 0.0879 | 0.1457 | 0.1660 |
| Akaike information criterion (AIC) | -19.9171 | -35.9292 | -29.8650 | -28.2998 |
| modified Akaike information criterion (AICc) | -15.9171 | -31.9292 | -25.8650 | -24.2998 |

Fitted parameter, SSE, AIC and, AICc of saturated phagocytosing model with CAR-macrophage and Raji cell concentration (System 5, *Methods*) are given in Table S4. By comparing SSE, AIC and AICc, we chose saturated phagocytosing model (System 5, *Main text*) to describe CAR-macrophage mediated Raji cell phagocytosing kinetics.

**Table S4.** Estimated Parameters of CAR-Macrophage Phagocytosing Using the Saturated Phagocytosing Model (System 5*, Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Phagocytosing rate of CAR macrophage, | 9.3125 | 2.7452 | 5.7937 | 1.2684 |
| Saturation in phagocytosing efficiency as tumour cell concentration increases, | 5.1414 | 2.3628 | 4.13133 | 0.8918 |
| Saturation in phagocytosing efficiency as CAR-macrophage/ normal macrophage concentration increases, | 4.7167 | 0.8490 | 2.02086 | 0.4789 |
| Sum of squared error (SSE) | 0.0978 | 0.0185 | 0.0072 | 0.0311 |
| Akaike information criterion (AIC) | -32.6485 | -52.6304 | -63.9546 | -46.3971 |
| modified Akaike information criterion (AICc) | -20.6485 | -40.6304 | -51.9546 | -34.3971 |

Fitted parameter and SSE of saturated phagocytosing model with normal macrophage and Raji cell concentration are given in Table S5.

**Table S5.** Estimated Parameters of Normal Macrophage Phagocytosis Using the Saturated Phagocytosis Model (System 5*, Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Phagocytosing rate of CAR macrophage, | 0.000214 | 0.000355 | 1.048544 | 0.91454 |
| Saturation in phagocytosing efficiency as tumour cell concentration increases, | 0.000348 | 0.000597 | 1.695939 | 1.54100 |
| saturation in phagocytosing efficiency as CAR-macrophage/ normal macrophage concentration increases, | 0 | 0 | 0.013615 | 0 |
| Sum of squared error (SSE) | 0.0635 | 0.0295 | 0.0256 | 0.0274 |

Fitted parameter of saturated phagocytosing model with extra CAR-macrophage death due to phagocytosing Raji cells (System 6*, Methods*) are given in Table S6.

**Table S6.** Estimated parameters of saturated phagocytosing model with extra CAR-macrophage death (System 6*, Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Phagocytosing rate of CAR macrophage, | 1.3874 | 5.3402 | 11.1890 | 10.3154 |
| Saturation in phagocytosing efficiency as tumour cell concentration increases, | 0.7660 | 4.59647 | 7.97854 | 7.2527 |
| Saturation in phagocytosing efficiency as CAR-macrophage/ macrophage concentration increases, | 0.7027 | 1.6516 | 3.90274 | 3.8947 |
| Death rate of CAR-macrophage cell due to phagocytosing Raji cells, | 0 | 0 | 0 | 0 |

1. **CAR-Macrophage Kinetics with Raji Cells**

In the main text, we demonstrated that CAR-macrophage can induce bistable Raji cell kinetics. Here, we provided CAR-macrophage kinetics in Fig. S2.

A graph of a patient's growth

Description automatically generated with medium confidence

**Figure S2. Simulated Kinetics of CAR-Macrophage with Varying Tumour Burdens.** This figure presents the simulated kinetics of CAR-macrophages across different tumour burdens, as shown in panels (A), (B), and (C). The purple, green, red, and blue curves illustrate the kinetics for both Raji cells and CAR-macrophages corresponding to tumour burdens of 1×106 cells/ml, 3×106 cells /ml, 5×106 cells/ml and 7×106 cells /ml, respectively. Since the CAR-macrophage concentrations were kept consistent across these simulations, their corresponding curves largely overlap.

In the main text, we presented the Raji cell and CAR-macrophage kinetics for a 30-day period following either a single 10 x 105 cells/ml infusion or a split infusion. To provide a more comprehensive view, we've included the CAR-macrophage kinetics for a 60-day duration in Fig. S3.

A graph showing the results of a treatment

Description automatically generated with medium confidence

**Figure S3. Simulated CAR-Macrophage Kinetics Over 60 Days with Varying Tumour Burdens.** This figure displays the simulated kinetics of CAR-macrophages over a 60-day period, across different tumour burdens, as presented in panels (A) and (B). The purple, green, red, and blue curves illustrate the CAR-macrophage kinetics corresponding to tumour burdens of 1×106 cells/ml, 3×106 cells /ml, 5×106 cells /ml and 7×106 cells /ml, respectively. Since the initial CAR-macrophage concentrations were kept consistent across these simulations, their respective curves largely overlap.

**Reference**

1. Wang, Q., et al., *Effect of adenovirus-mediated p27 gene expression on the proliferation and apoptosis of HL-60 and Raji cell lines.* Hematol J, 2004. **5**(6): p. 519-23.

2. Liu, M., et al., *CAR-Macrophages and CAR-T Cells Synergistically Kill Tumor Cells In Vitro.* Cells, 2022. **11**(22).