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

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3. **Parameter estimation**

In this section, we will first describe the estimation of Raji growth kinetics in absence of CAR macrophage/ macrophage with logistic model (System 1, Methods). Next, we describe the estimation of CAR macrophage killing parameter with unsaturated killing model (System 2, Methods), semi-saturated killing model with increase of Raji cell concentration (System 3, Methods), semi-saturated killing model with increase of CAR macrophage cell concentration (System 4, Methods), saturated killing model (System 5, Methods) and saturated killing model with extra macrophage death (System 6, Methods).

* 1. Estimation of Raji growth kinetics in absence of CAR macrophage/ macrophage

In this section, we fit logistic model to Raji proliferation assay data provided in Reference [1]. We found that per capita growth rate , maximum Raji capacity and sum of squared error (Cells/ ml/day). 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 provided in Reference (1).** Red cycle is experiment data, and blue point and curve is estimated value.

* 1. Estimation of CAR-macrophage/ macrophage killing parameter with unsaturated killing model, semi-saturated killing model and saturated killing model

In this section, we will describe the estimation of CAR-macrophage killing parameter with unsaturated killing model (System 2, *Methods*), semi-saturated killing model with increase of Raji cell concentration (System 3, *Methods*), semi-saturated killing model with increase of CAR macrophage concentration (System 4, *Methods*), saturated killing model (System 5, *Methods*) and saturated killing model with extra macrophage death (System 6, *Methods*). Reference [2] provided three biological replicates of CAR-macrophage cytotoxicity assay data, and we used their geometric mean as fourth biological replicates.

First, fitted parameter, sum of squared error (SSE), Akaike information criterion (AIC) and modified Akaike information criterion (AICc) of unsaturated killing model are given in Table S1.

**Table S1.** Estimated parameters of CAR-macrophage killing using unsaturated killing mode (System 2*, Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| killing 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 parameter, SSE, AIC and AICc of semi-saturated killing model with increase of Raji cell concentration are given in Table S2.

**Table S2.** Estimated parameters of CAR macrophage killing using semi-saturated killing model with increase of Raji cell concentration (System 3, *Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Killing rate of CAR macrophage, ,  (mL/cells/day) | 4.8868 | 4.8868 | 5.0273 | 5.0747 |
| Saturation in killing 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 parameter, SSE, AIC and AICc of semi-saturated killing model with increase of CAR-macrophage concentration are given in Table S3.

**Table S3.** Estimated parameters of CAR macrophage killing using semi-saturated killing model with increase of CAR macrophage concentration (System 4, *Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Killing rate of CAR macrophage, ,  (mL/cells/day) | 495.2441286 | 279.5276567 | 327.1255544 | 262.61057 |
| Saturation in killing efficiency as CAR-macrophage/ 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 killing model with increase of CAR-macrophage and Raji cell concentration are given in Table S4. By comparing SSE, AIC and AICc, we chose saturated killing model (System 4, *Main text*) to describe CAR-macrophage mediated Raji cell killing kinetics.

**Table S4.** Estimated parameters of CAR macrophage killing using saturated killing model (System 5*, Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Killing rate of CAR macrophage, | 9.3125 | 2.7452 | 5.7937 | 1.2684 |
| Saturation in killing efficiency as tumour cell concentration increases, | 5.1414 | 2.3628 | 4.13133 | 0.8918 |
| Saturation in killing efficiency as CAR-macrophage/ 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 killing model with increase of macrophage and Raji cell concentration are given in Table S5.

**Table S5.** Estimated parameters of macrophage killing using saturated killing model (System 5*, Methods*)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Biological replicates 1 | Biological replicates 2 | Biological replicates 3 | Biological replicates 4 |
| Killing rate of CAR macrophage, | 0.000214 | 0.000355 | 1.048544 | 0.91454 |
| Saturation in killing efficiency as tumour cell concentration increases, | 0.000348 | 0.000597 | 1.695939 | 1.54100 |
| Saturation in killing efficiency as CAR-macrophage/ 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 killing model with extra CAR-macrophage death due to killing Raji cell are given in Table S6.

**Table S6.** Estimated parameters of saturated killing model with extra CAR-macrophage death (System 5*, Methods*)

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

1. **CAR-macrophage kinetics with Raji cell**

In section CAR-macrophage induced bistable tumour kinetics, main text, we exhibited that CAR-macrophage can induce bistable Raji cell kinetics. Here, we provide 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 different combinations of pre-infusion tumour burden (A), (B) and (C).** Purple, green, red and blue curves represent Raji cell and CAR macrophage kinetics with pro-infusion tumour burden 1×106 cells/mL, 3×106 cells /mL, 5×106 cells /mL and 7×106 cells /mL in (A–C). Because initial CAR-macrophage concentration are same, purple, green, red and blue curves overlap.

In main text, we exhibited Raji cell kinetics and CAR-macrophage kinetics with 10 x 105 cells/ml single infusion or split infusion for thirty days. Here, we provided CAR-macrophage kinetics for sixty days in Fig. S3.

A graph showing the results of a treatment

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**Figure S3.** **Simulated kinetics of CAR-macrophage with different combinations of pre-infusion tumour burden for sixty days (A) and (B).** Purple, green, red and blue curves represent CAR macrophage kinetics with pro-infusion tumour burden 1×106 cells/mL, 3×106 cells /mL, 5×106 cells /mL and 7×106 cells /mL in (A–B). Because initial CAR-macrophage concentration are same, purple, green, red and blue curves 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).