Whack-a-Mole

NMU-China iGEM

iGEM 2022

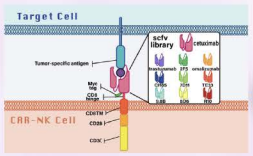
—aim for a billion targets, strike with a giant hammer

key point

- ★ A novel therapy to tackle tumor heterogeneity
- ★ A combination of antibody library technology and chimeric antigen receptor technology
- ★ Putting clonal selection theory into practice
- ★ A vivid interpretation of Whack-a-Mole game in the life science

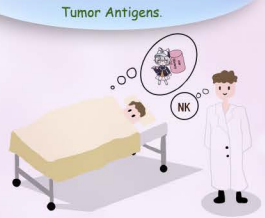
Abstract

When we play **Whac-a-Mole**, we keep hitting them with our little hammers, but they always pop up on the other side of the machine, like neoantigens that are constantly appearing as tumors grow and evolve. To address this problem, we conceived and preliminarily tested the **CAR-NK92 library therapy** based on antibody library technology.



According to clonal selection theory, a healthy adult can produce antibodies against all antigens,including Tumor Antigens.

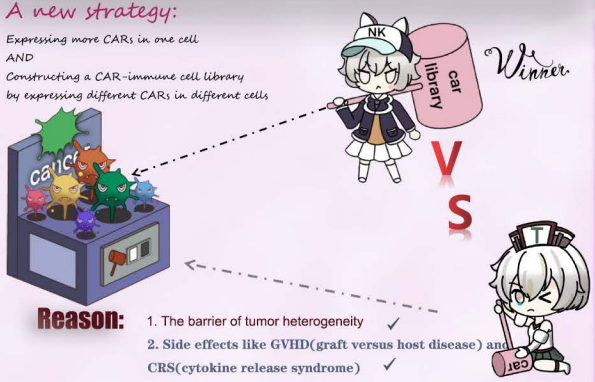
We have a conjecture that mRNAs encoding antibodies extracted from the peripheral blood of healthy humans, can construct the antibody library after negative selection, and the CAR-NK92 library consisting of NK-92 cells loading the antibody library can target all tumor antigens.



Background

A new strategy:

Expressing more CARs in one cell AND Constructing a CAR-immune cell library by expressing different CARs in different cells



Reason:

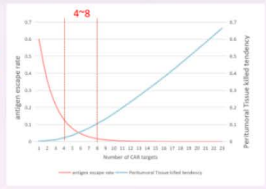
1. The barrier of tumor heterogeneity ✓
2. Side effects like GVHD(graft versus host disease) and CRS(cytokine release syndrome) ✓

Modeling

1.Limitations on the number and types of CARs that can be expressed in a single cell

We derived the relationship between the number of types of CARs expressed by individual immune cells and the rate of antigen evasion and over-misfiring of normal cells.

How? **Method:** Bioinformatics analysis



Result: Taking colon cancer cells as an example, the number of CAR types should be controlled within 4-8 types. And related research has shown that transfection of multiple CARs on a single cell is challenging and expression of multiple CARs on a single cell is prone to ligand non-dependent tonic signaling.

Decision: To express multiple CARs on a large number of immune cells!

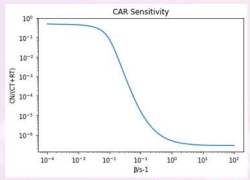
2.Expressing multiple CARs on a large number of immune cells

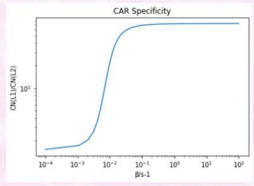
2.1 CAR-NK92 suicide gene pathway feasibility model

Method: Improved CAR activation model, based on the original McKeithan kinetic proofreading model

$$\frac{C_N}{C_T + R_T} = \frac{M}{M + K_D} \theta^N \frac{1 - \frac{\alpha M}{\beta K_D}}{1 - \frac{\alpha M}{\beta K_D}}$$

Result: The biological properties of CAR-NK92 cells in terms of sensitivity, specificity and time efficiency in the recognition of exogenous molecules were reflected more successfully and realistically.It has implications for predicting the activity of CAR-NK92 cell gene circuits in animal and clinical experiments.





2.2 AP1903 pro-apoptosis model

Method: Optimize the linear model equation and obtain a relatively complete effect change equation to describe the relationship between efficacy, time and concentration of AP1903.

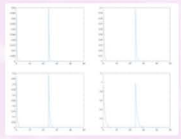
$$E = k_1 \times C_p + E_0$$ the linear model equation

$$E = E_0 \pm \frac{E_{max} C_p}{C_{v\,max} + C_p}$$ a relatively complete effect change equation

Result:

★ When the initial concentration was set at 0.05, 0.1, 0.5 and 1.0mg/kg and AP1903 was injected at an interval of 12h, the relationship of plasma pharmacodynamics with time was observed.

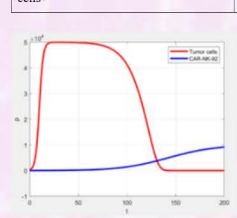
★ When the initial concentration was set at 0.05, 0.1, 0.5 and 1.0mg/kg and AP1903 was injected at an interval of 24h, the relationship of plasma pharmacodynamics with time was observed.



2.3 CAR-NK92 proliferation model

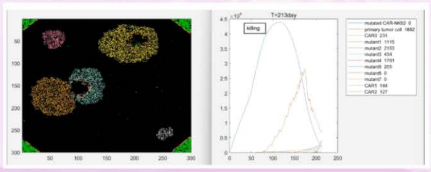
Method: Build Lotka-Volterra predator-prey model, importing logistic elements.

Tumor cells:	CAR-NK-92:
$\dot{x}(t) = rx(1 - \frac{x}{N_1} - \frac{a}{r}y)$	$\dot{y}(t) = dy(-1 - \frac{y}{N_2} + \frac{b}{d}x)$
r:growth rate of tumor cells	d:CAR-NK-92 apoptotic rate
a:ability of CAR-NK92 to kill tumor cells	b:ability of tumor cells to induce CAR-NK92 enrichment



Picture indicates how their numbers change over time!

3.Cellular automata visualization simulation



Visual dynamic simulation of the CAR-NK92 library competing with tumour cells

HP

Pre-project Phase

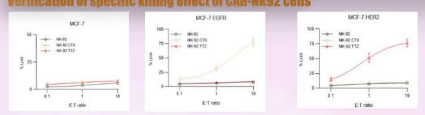
General survey questionnaire and interview → Interview the doctor

Implementation Phase

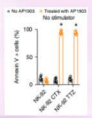
Invite an expert → Publicize the project and Synthetic Biology → Follow up with previous interviewees → Educate high school students → Take the advice of ZJUiint → Invite the professor → Cooperate with ZJUiint on modeling

Result

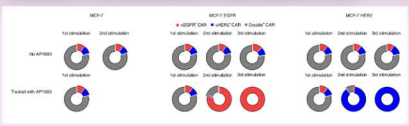
Verification of specific killing effect of CAR-NK92 cells



Verification of the ability of AP1903 to cause apoptosis when the engineered NK cells are not stimulated



Verification of the growth dynamics of logic-gated NK-92 cells



The gene circuit was effective in the cell-based CAR screening method and adding AP1903 was essential for the enrichment of CAR NK-92 cells.

In these experiments, we determined the kinetics of the genetic circuit in which NFAT was coupled with KRAB activation in engineered NK-92 cells after CAR stimulation !

Design

Design thought

How can we deal with the large number of tumor antigens ?

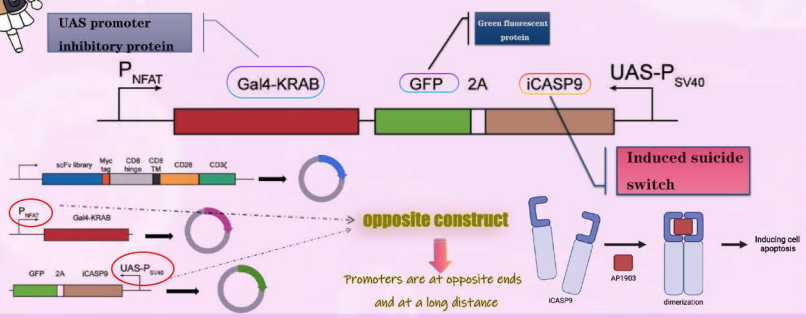
Engineered CAR-immune cells

Design plan

Selection of chassis cells → Kill switch circuit

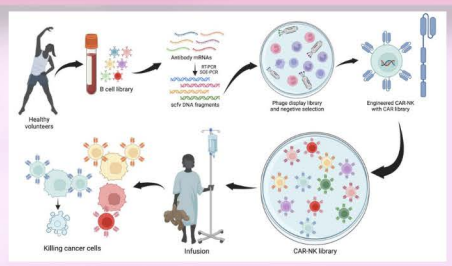
NK-92 cell line — Our best candidate

1. The **proliferation rate** of the NK92 cell line is quite impressive.
2. CAR-NK cells do not cause **graft-versus-host responses** and **CRS**.



NOTE: Engineering Optimization

Future Prospect



Despite the long way to go from experiment to clinic, we make a preliminary outlook on the future of this project!

1. Draw 200 copies of peripheral blood from healthy volunteers.
2. Extract mRNAs encoding antibodies in B cells and use RT-PCR and SOE-PCR to form a scFv cDNA library.
3. Select leukocytes from volunteers on a large scale and obtain their HLA antigens as material for negative selection.
4. After negative selection, reserve the negative ones to form the final library that can be put into use.
5. Before the patient is treated, confirm that the scFv library has no affinity to the patient's HLA antigens.
6. Construct CAR-NK92 cells carrying the scFv library and infuse them into the patient for treatment.