A single-cell omics-informed virtual trial framework for synNotch-gated CAR-T cells

Aaron Lee [1] Charissa Wren [2]

[1]Department of Computer Science, University of Warwick, Coventry, United Kingdom [2]School of Life Sciences, University of Warwick, Coventry, United Kingdom

Introduction

Tumour cells express tumour-associated antigens (TAAs). CAR-T cells are immune cells engineered to express chimeric antigen receptors (CARs), which bind TAAs to trigger cancer cell killing.
Trials have shown these to be highly effective in haematological cancers, however they have had much less success in solid tumour treatment due to antigen heterogeneity and on-target, off-tumour toxicity [6].
Next-generation CAR-T cells improve specificity by using engineered genetic circuits to target multiple TAAs.
SynNotch receptors must bind two TAAs for activation (AND gating), thus enhancing targeting precision.

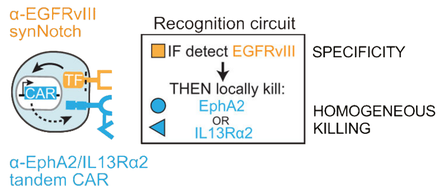

Figure 1. **synNotch tanCAR**. Credit Choe et al. 2021

Tandem CARs activate upon binding any target TAAs (OR gating), thus helping to deal with antigen heterogeneity-associated issues such as antigen escape [11].
Clinical trials are so far limited and so predicting optimal design and dosing for gated CAR-T cells remains difficult. We therefore present an integrated pharmacodynamics model and virtual trial framework which simulates gated CAR-T therapies. Virtual patients are initialised expressing both inter-patient and intra-tumoural antigen heterogeneity, derived from single-cell transcriptomic data.

scRNAseq Pipeline

Datasets

Public single-cell RNA sequencing (scRNAseq) data of breast cancer biopsies obtained from GSE176078 [13].

Data processing

1. Quality control. 2. Cell type annotation and assessment of existing annotations. 3. Subsetting surfaceome genes. 4. Imputation of zero values. These were explored alongside non-imputed values.

Normal reference data

Samples from 31 tissues were integrated as a reference dataset.

Identification of ideal target combinations

Gene combinations differentiating the most between malignant and non-malignant cells were highlighted as potential targets for AND gating, reapplying an existing approach to samples from individual patients [7].

Target expression

Expression of identified targets were explored, assessing their suitability as CAR-T targets. A brief literature review was carried out to evaluate their suitability and current potential as targets.

Model initialisation

Distributions of gene expression for target antigens were extracted for model initialisation.

Model derivation

Tumour population

$$\frac{\partial n}{\partial t} = D_n \nabla^2_{x,y} n + r\left(1 - \frac{N_{\text{total}}}{N_{\max}}\right) n + M - e\frac{n}{n + K_n} n_{\text{TA}}$$

$n(\mathbf{a}, x, y, t)$ is density of tumour cells expressing the antigen profile $\mathbf{a}$. The equation models logistic growth, mutational antigen loss ($M = \int_{\mathbb{R}^d} \left[m(\mathbf{a}^{(i)}, \mathbf{a}) \cdot n(\mathbf{a}^{(i)})\right] \, \mathrm{d}^d\mathbf{a}^{(i)}$), and T-cell effected cell death [9].

CAR-T cell populations

$$\frac{\partial n_{\text{TN}}}{\partial t} = D_{\text{TN}} \nabla^2_{x,y} n_{\text{TN}} - \int_{\mathbb{R}^d} \left[T_A - T_I + T_{O,N}\right] \, \mathrm{d}^d\mathbf{a} - l_{\text{TN}} n_{\text{TN}}$$

$$\frac{\partial n_{\text{TA}}}{\partial t} = D_{\text{TA}} \nabla^2_{x,y} n_{\text{TA}} + r_{\text{TA}} \frac{n}{n + K_r} n_{\text{TA}} + T_A - T_I - T_{O,A} - l_{\text{TA}} n_{\text{TA}}$$

$$T_A = k_A \cdot g_A([\text{CAR}]) \cdot \frac{n}{n + K_A} n_{\text{TN}}$$

CAR T-cell populations are divided into active, $n_{\text{TA}}(\mathbf{a}, x, y, t)$ and inactive, $n_{\text{TN}}(x, y, t)$. Active T cells are considered bound to tumour cells expressing the antigen profile $\mathbf{a}$. T functions represent transfers between active/inactive and apoptosis depending on circuit architecture.

Gene circuits

$$\frac{\partial [\text{CAR}_{\boldsymbol{\alpha}}]}{\partial t} = \frac{n}{n + K_A} f([\text{TF}]) \frac{1}{1 + \exp\left(-(a - \beta)/\varepsilon\right)} - k[\text{CAR}_{\boldsymbol{\alpha}}]$$

Where $[\text{CAR}_{\boldsymbol{\alpha}}]$ is the per CAR-T cell concentration of CAR. $\boldsymbol{\alpha}$ represents the set of antigens able to bind the (tandem) CAR.

Multi-input AND gates can be implemented via synNotch chaining [11]. This can be represented by recursion, replacing the output of the equation with a [TF], which is then used as the inputs to $f([\text{TF}])$ of $\frac{\partial [\text{CAR}_{\boldsymbol{\alpha}}]}{\partial t}$.

The model is simulated computationally using Julia.

Results

Combinatorial CAR-T targets

- Of the samples explored, the target antigens were often consistent with previously suggested therapeutic targets, including some already tested as CAR-T candidates (Table 1).

Sample ID	Targets	Target Status
CID4471	BCAM	Suggested as a target in breast cancers [1].
	CD151	Some drugs targeting CD151 have already been suggested. In silico study suggests potential drugs that target CD151 to treat breast cancer and glioblastoma [12][4].
CID4495	**CD24**	Previously tested as potential CAR-T target in triple negative breast cancer [14].
	EPCAM	Undergoing testing as a CAR-T target in trials [3][8].
CID3963	**BST2** (CD317)	CAR-T cells targeting BST2 have been successfully tested for the treatment of glioblastoma [5]
	CD74	Often highly expressed in breast cancers, and suggested as a therapeutic target [10]. Also tested as a CAR-T target in mantle cell lymphoma models [2].

Table 1. **Indicated combinatorial CAR-T targets in breast cancer.** Those already existing as CAR-T targets have been highlighted in bold. A subset of samples have been presented.

Heterogeneity in target expression

- Within samples, variation in target expression was seen, with cells expressing neither, one or both of the targets across varying levels (Figure 2).
- Across samples, inter-patient variability lead to different optimal combinatorial targets. However, some overlap was seen, with some targets, in particular CD24, appearing multiple times (Table 1).

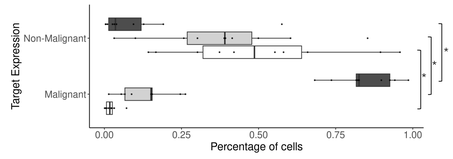

Figure 2. **Target expression across samples in malignant and non-malignant cells.** Dark grey: co-expression of targets; grey: one target expressed; white: neither target expressed. All expression categories differed significantly between malignant and non-malignant cells (Wilcoxon signed rank test, $p < 0.05$). Binarised counts were used.

Co-expression of targets are reduced in non-malignant cells

- As expected, expression of target antigens were seen in both malignant and non-malignant cell types (Figure 2), indicating that gated CAR-T approaches may be applicable.
- Target expression varied significantly between malignant and non-malignant cells. In particular, co-expression of target antigens was significantly lower in non-malignant cells (Wilcoxon signed rank test, $p < 0.001$).
- However, some samples retained co-expression in non-malignant cells, suggesting potential for on-target, off-tumour effects.

Predicted differences in tumour response

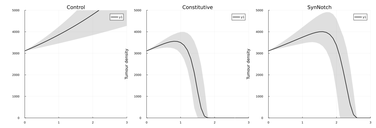

Figure 3. **Predicted tumour size over time for control, constitutive CAR, and synNotch-regulated CAR.** Ribbon represents range across virtual patient cohort. Antigen distributions derived from patients CID44971, CID4495, CID4066, and CID3963.

- The model successfully predicts differences in tumour response with treatments reducing tumour size. SynNotch-regulated CAR T-cells are predicted to effect a slower but still efficacious response (Figure 3).
- Whilst slower responses are seen, this trade-off allows for reduced on-target, off-tumour effects, as demonstrated by the reduced co-expression of these targets in non-malignant cells (Figure 2).
- Moderate variation is seen across virtual patients, though larger variation might be expected if additional pharmacokinetic parameters were varied.

Consistency across patients

- Treatment efficacy was robust to inter-patient variability in target expression and results were consistent across antigen combinations in different patients.

Discussion

Summary

- Here, a framework is presented which can model the tumour response of a cohort of virtual patients treated with gated CAR-T cell therapies. It can simulate arbitrary logic circuits, involving synNotch (AND), tandem CARs (OR), iCARs (NOT), and OFF-Notch (NOT).
- The presence of sample-specific and heterogeneous antigen expression was confirmed, highlighting the potential utility of personalised gated therapies.
- Single-cell gene expression data facilitated the identification of suitable targets, which were used to inform the framework under which patient-specific responses were explored.
- Overall, our model predictions consolidate theories of synNotch-gated CAR-T cells being an effective means to combat tumour antigen heterogeneity.

Limitations

- Gene expression profiles may deviate from true values, especially due to dropouts seen in single cell data. Additionally, gene expression may not directly correspond to protein levels.
- Single biopsies may not be representative of the total heterogeneity within a tumour.
- The model is theoretically able to be initialised with patient-specific spatial data, though due to a lack of such data available we simulate a uniform random spatial distribution.

Further directions

- Inclusion of PKPD in virtual patients to inform optimal dosing, and analysis of factors such as improved persistence of synNotch-gated CAR-T cells.
- Parameter optimisation. There is a lack of available data to accurately estimate a number of our parameters. Future efforts may include *in vitro* assays to quantify circuit-specific parameters.
- Protein level validation of target expression and incorporation of patient-specific normal reference tissues to inform appropriate targets.
- Prediction of on-target, off-tumour effects by simulating healthy tissue cell populations, to supplement the assessment of off-target effects explored in the single-cell data.

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