Computational Algorithms and Large-Scale Data for CAR T Cell Therapy Resistance

CAR T cell therapy has already broken new ground in treatment for cancer patients, and large-scale omics data integration and computational analyses are bound to shape the new generation of CAR T cells

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Chimeric antigen receptor T (CAR T) cells have become an important and novel therapy for patients with B cell malignancies, mainly due to recent advances in gene, protein, and cell engineering. To date, CAR T cell therapy has been successful in patients with large B cell lymphoma (LBCL) and acute lymphoblastic leukaemia, who show complete remission after a single infusion of CAR T cells. However, the success of CAR T cell treatment in solid tumours has been minimal. Additional consideration of the factors that shape the response and resistance is needed for driving the development of next-generation CAR T cell therapies to increase efficacy in both haematological malignancies and solid tumours. This review will discuss the major factors that result in resistance to CAR T cell therapy and how data-driven computational approaches can help identify genes, proteins, and small molecules that can boost engraftment, expansion, and persistence of CAR T cells.

CAR T Cell Design: The Fundamentals

CAR T cells are engineered by the fusion of the variable region of the heavy and light chains of a monoclonal antibody to a transmembrane region and an intracellular domain via a hinge (see **Figure 1**). The variable region of the monoclonal antibody provides specificity to the tumour-associated antigen,

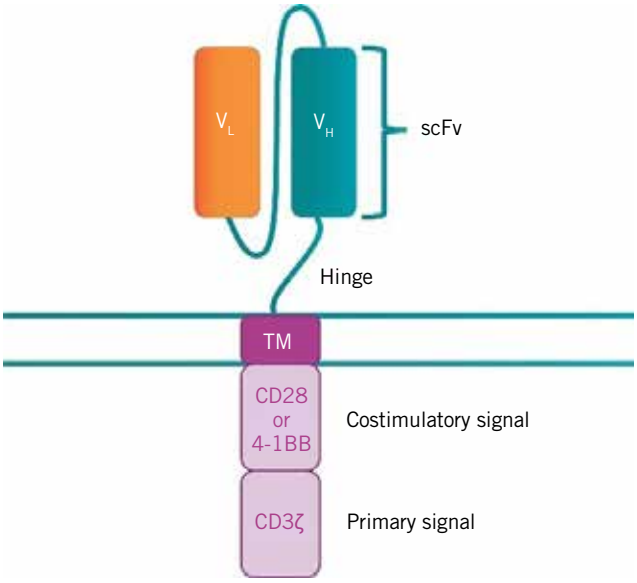

Figure 1: Structure of a second-generation chimeric antigen receptor, which is engineered with the single chain of variable fragment (scFv) of the light and heavy chain of a monoclonal antibody and fused to transmembrane, costimulatory, and CD3ζ domains. Both primary and costimulatory signals (i.e., CD28 or 4-1BB) are essential to trigger an efficient cytolytic CAR T cell response. V_L: light chain, V_H: heavy chain, TM: transmembrane domain

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while the intracellular domain induces the cytolytic response distinctive of CAR T cells. In more detail, the intracellular domain contains the CD3-ζ region fused to a costimulatory domain such as CD28 or 4-1BB (1-3). These provide efficient activation of CAR T cells since both a primary and costimulatory signal are triggered. Differences can be found between CD28 and 4-1BB costimulatory signalling used in the CAR T cells. For instance, the incorporation of CD28 domain leads to quicker and higher T cell expansion, but these cells do not persist beyond two months (4-6). Alternatively, the fusion with 4-1BB endodomain shows lower peak and slower expansion, but cells can persist for years (6-8). However, there is no clear indication that rapid and intense expansion will result in a greater anti-tumour response. Therefore, the optimal costimulatory domain for CAR T cells for both haematological malignancies and solid tumours will need to be explored further to determine the most effective treatment.

Resistance to CAR T Cell Therapy

One of the major issues in using engineered CAR T cells for cell therapy is an intrinsic dysfunction known as T cell exhaustion. This can be induced by both antigen-independent signalling from CAR T cell receptor aggregation, as well as excessive stimulation triggered by the high tumour burden (9). This is exemplified by Steven J Schuster *et al*, who showed that non-responding patients with LBCL have higher expression levels of exhaustion markers on CD19 CAR T cells in the tumour sites when compared with patients with complete response (10). Moreover, it has been demonstrated that the expression of T cell exhaustion markers in the initial cell apheresis and manufactured CAR T cell product are themselves predictive of non-response in clinical trials in patients with chronic lymphoblastic leukaemia.

Extrinsic factors can also influence the resistance to CAR T cell therapy. The tumour microenvironment (TME) plays an important role as an immunosuppressant of CAR T cell function, either by producing cytokines that directly suppress function or by competing for nutrients that are essential for the function of effector T cells. Several studies using animal models have focused on the development of armoured CAR T cells, which constitutively secrete cytokines that should enhance T cell expansion and anti-tumour immunity by the transduction of genes such as IL-12, IL-15, and IL-18. These armoured CAR T cells are currently being translated

Issue to be solved	How will computational methods help solve the issue?	Example and references
Cell exhaustions	Identify new genes, transcriptions factors, and proteins that overcome exhaustion	c-Jun overexpression has increased the persistence of CAR T cells (18)
TME microenvironment suppression	Help designing switch receptors to convert suppressor signals to activation signals	Switch receptor that activate CD28 costimulatory signal upon ligation of PD-1 (19)
Tumour penetration	Design of small molecules that help in the induction of chemokine receptors to improve tumour infiltration	Induction of CCR9 in CAR T cell by retinoic acid could increase gut homing receptor in CAR T cells for colorectal cancer (20)

Table 1: How to overcome resistance to CAR T cell therapy

into the clinic (11-13). However, it is possible that this transduction of multiple genes may affect the safety of the product, and, therefore, alternative mechanisms to induce enhancer or inhibit suppressor cytokines should be explored.

Although the migration of CAR T cells to solid tumours has been observed, such as with brain tumours, they have shown limited killing capacity, which could be associated with the reduced number of CAR T cells that infiltrate the tumour. There are several preclinical studies showing that the induction of chemokine receptor expression on T cells leads to increased cell migration and better tumour engraftment. An example of this is the transduction of CCR2 in mesothelin CAR T cells, which increases tumour infiltration and anti-tumour efficacy (14). This suggests that alternative methods to induce chemokine receptors are needed to overcome resistance to tumour engraftment.

How Analysis of Large-Scale Datasets Will Overcome Resistance and Enhance the Efficacy and Engraftment of CAR T Cells

Next-generation sequencing and high-throughput data approaches will play a key role in the identification of gene regulators or chemicals that can prolong the persistence of CAR T cells (see **Table 1**). For example, datasets such as the FANTOM5 consortia data have been employed in new approaches, using a Big Data algorithm to compare gene expression and identify the optimal combination of transcription factors required to directly convert any cell type into any other (15). Additionally, the same dataset has made it possible to identify enhancers and promoters that are important in T cell and macrophage differentiation by profiling human T cells and monocytes (16-17). This type of large-scale data could be able to identify regulatory molecules needed to overcome resistance in CAR T cells. An example of using next-generation sequencing for inhibiting exhaustion in CAR T cells is the work done by Rachel C Lynn *et al*. A combined analysis of transcriptomics and ATAC-seq data (assay for transposase-accessible chromatin using sequencing) was carried out in different subtypes of CAR T cells, and c-Jun was identified as an important factor to overcome exhaustion in CD8 T cells. It was shown that c-Jun overexpression, in a CD19-CD28CD3ζ CAR T cell model, induced expansion of CAR T cells, resulting in an increase of function and reduction in the terminal differentiation (exhaustion) (18). These types of approaches can also be applied to identify and engineer switch receptors that convert or transform suppression signals and increase CAR T cell resistance to the TME. This includes the design of dominant-negative forms of suppressive receptors, such as IL-10 or TGF-β receptors, or switch receptors that activate CD28 in response to PD1 ligation (19).

Computational approaches could be used to improve CAR T cell tumour penetration by aiding the design of small molecules and identifying metabolites to trigger the expression of chemokine receptors and integrins. For example, retinoic acid, a product derived from vitamin A, has been shown to induce the integrin α4β7 and the chemokine receptor CCR9, both of which are important for the migration of T cells to the gut (20). Retinoic acid could, therefore, be used to induce gut homing receptor expression in EGFR CAR T cells for colorectal cancer, with the expectation of improving CAR T cell infiltration into the tumour. Another example is to have small molecules that could induce endogenous CCR2 expression, as an alternative for the transduction of CCR2 gene in mesothelin CAR T cells (14). This could increase the safety of CAR T cell therapy by eliminating any risks related to the insertion of the CCR2 gene.

In conclusion, large-scale omics data integration and computational analyses are bound to shape the new generation of CAR T cells, improving engraftment, expansion, and persistence by identifying novel genes, proteins, and transcription factors. Several international efforts are being carried out to profile the human immunome at an unprecedented resolution, including using single-cell profiling. In the coming years, artificial intelligence and computer algorithms could be used to design small molecules and switch receptors that overcome exhaustion, making CAR T cells resistant to the suppressive TME and improving the tumour penetration in both haematological malignancies and solid tumours.

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