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Recent Improvements in Gene Regulations and Immunology

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Abstract

The possibility of employing genetically altered cells as therapeutic agents have been firmly proven by the effectiveness of Chimeric Antigen Receptor (CAR) T cell treatment against haematological tumours. Even while cell therapy has made significant strides, its entire range of advantageous uses is yet unknown. The ability to apply genetic control circuits, which permits varied signal detecting and logical processing for the best response in the intricate tumour microenvironment, is one of the special benefits of cell treatments. We will first discuss design factors for cell therapy control circuits that meet clinical requirements from this angle. After contrasting and comparing important aspects of some of the most recent advancements in control circuit design, we'll talk about possible future paths.

Keywords: Cell, Gene, Immune, Therapies.

1 | Introduction

Cells are highly developed information-processing systems that can perceive environmental cues, carry out intricate calculations, and generate outputs, including gene expression, signalling molecules, morphological changes, and cell proliferation [1]. Additionally, various cell types have developed unique traits that enable them to thrive in diverse settings and carry out a range of functions [2].

These characteristics make cells great options for intelligent medicines that have higher levels of safety and effectiveness. Indeed, a variety of cell types, including bacteria and stem cells, have been investigated for the creation of cell treatments [2].

Specifically, the human immune cell is one of the most significant cell groups for creating therapies [3]. Five FDA-approved therapeutics for B cell malignancies have resulted from T cells being genetically modified with

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a Chimeric Antigen Receptor (CAR). These cells have shown strong anti-cancer cytotoxicity in clinical settings (FDA, U.S., 2021a, 2021b, 2017a, 2020, 2017b).

Immune cell treatments are promising, but before we can fully utilize them, several issues must be resolved. The toxicity brought on by the modified immune cells' off-tumour targeting and overactivation is one of the most urgent issues with cellular immunotherapy [2].

Furthermore, a dynamic intervention is required instead of a static, one-time treatment due to the variability and ongoing evolution of many illnesses [4], [5]. Furthermore, the difficulties CAR T cells have in fighting solid tumours demonstrate the need for additional advancements in immune cell therapy effectiveness.

To solve these problems, more precise and controlled advanced therapeutic cell designs are required. Most crucially, to develop successful medicines, the issues regarding safety and efficacy must be resolved together.

2 | Literature Review

2.1 | Cell-Autonomous Versus Exogenous Control: Design Considerations

Cell treatments, in contrast to most other therapeutic methods, may be fitted with complex gene circuits to increase their safety, effectiveness, and targeted specificity. Gene circuits come in different forms, but they may be generally divided into two groups: external control and cell-autonomous control (*Fig. 1*).

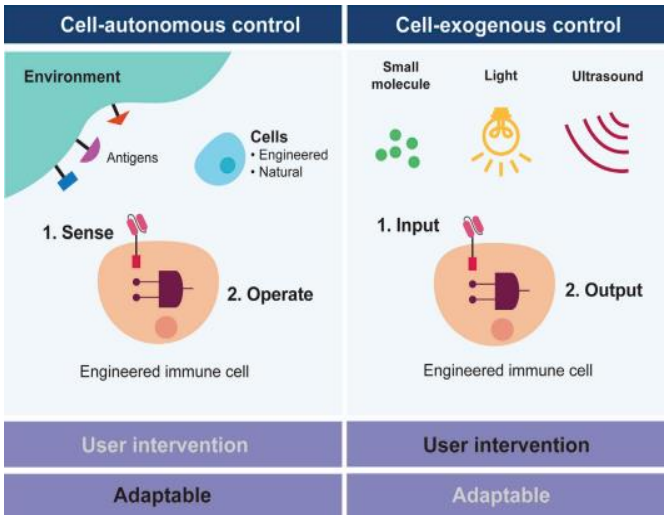

Fig. 1. Comparison between cell-autonomous and exogenous control.

[External control permits user interaction of the designed cells through inputs; autonomous control of engineered immune cells allows the cells to recognize and react to the environment or internal signals].

Signals from the original environment or from within the modified immune cells are used by cell-autonomous control gene circuits. Exogenous control gene circuits, on the other hand, are dependent on inputs from outside agents like light, ultrasonography, or tiny chemicals.

These circuits can be used in tandem and are not mutually exclusive. The relative advantages and disadvantages of each gene circuit should be considered when choosing to use gene circuits to enhance immune cell treatments. The ability of cell-autonomous circuits to function without human interference makes them appealing.

This capability could be required because some features, like accurately detecting a tumor using a combination of molecular markers, cannot be controlled manually. However, as the development of autonomous vehicles has shown, there may be obstacles to a fully autonomous system that need oversight and modification. Unpredictability is not acceptable in healthcare settings. Therefore, it will be very desirable to be able to construct cell treatments under external control.

The selection of the input control is one of the most important factors for external control circuits. The input might be applied extremely locally, such with light or ultrasound, or systemically, like with a tiny molecule. Although small compounds are simple to deliver, they may be hazardous or have poor pharmacokinetic characteristics.

On the other hand, noninvasive and accurate spatiotemporal control is offered by light and ultrasound. However, it might not be feasible to provide the patient with constant light and ultrasound therapy, which would be necessary to maintain immune cell activity.

The groundbreaking work in this field has been noted in an earlier study by Lim and June [6]. We want to highlight some of the most recent advancements in genetic circuits for immune cell treatment here. This study will explore the advantages and disadvantages of these gene circuits. We will conclude by giving an overview of how gene circuits may contribute to the upcoming class of smart cell treatments.

2.2 | Cell-Autonomous Circuit for Therapeutic Immune Cells

Patient-derived input signals can be sensed and processed by cell-autonomous gene circuits. Gene circuits are capable of sensing several types of input signals, including intracellular cell states, the mix of antigens from target and healthy cells, and the tumour microenvironment. These circuits provide the modified immune cells with more accurate temporal and contextual responses by providing logic and feedback control.

2.3 | Receptor Logic Circuits

Given that there is frequently no one antigen that can be used to categorize cancer cells, combinatorial antigen recognition is the most sensible strategy to enhance tumour targeting and lower the possible toxicity of cancer cell therapy.

Three of the most sophisticated logic circuits-split, universal, programmable CARs (SUPRA CARs), synthetic Notch (synNotch), and Colocalization-dependent Latching Orthogonal Cage/Key proteins (Co-LOCKR) that have been applied to perform up *Fig. 1* will be highlighted among the various receptor logic circuits to immune cells [7–13].

Comparing exogenous control with cell autonomy Engineered immune cells under autonomous control are capable of sensing and reacting to internal signals or the environment, and exogenous control allows the user to manipulate the engineered cells using a variety of inputs, including 3-input AND, NOT, and OR logic [14–18].

2.4 | CAR Circuits

In a conventional CAR, an antigen-binding domain is fused to important intracellular signalling domains from costimulatory receptors (like CD28 or 4-1BB) and T Cell Receptors (TCR) (like CD3z or CD3ε). For a complete T cell response, signalling from the costimulatory receptor domain and TCR is required. Similar to this, intracellular signalling domains from inhibitory receptors have been used to block the signal from the conventional Activating CAR (aCAR) in the case of Inhibitory CARs (iCARs) [13], [18–22].

Creating distinct CARs with distinct antigen targets to serve as TCR, costimulatory, and inhibitory receptors is the fundamental design premise of a multi-input CAR logic circuit (*Fig. 2a*).

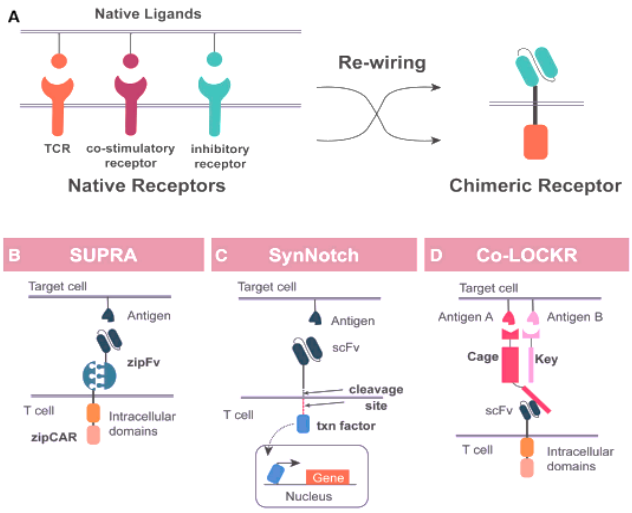

Fig. 2. Schematics of CARs and receptor logic circuits.

We see in *Fig. 3a* native receptors are the source of CARs created by swapping out their extracellular and intracellular domains to alter their targeting specificity.

Also *Fig. 3b* ZipFv and zipCAR make up SUPRA CAR. The same zipCAR can target different antigens by switching the zipFv, and in *Fig. 3c* response to the target antigen, the SynNotch receptor can stimulate gene expression. The membrane-bound transcription factor (txn factor) is released to stimulate gene expression after the antigen binds to the scFv domain.

In *Fig. 3d* CAR and the two adaptor proteins, Cage and Key, which make up the Co-LOCKR system. The cage domain can only be exposed to activate the CAR when the cage and key are coupled to the same target cell.

All in all, a distinct CAR is made for every signalling circuit. The endogenous signalling network will facilitate intracellular signal integration. The CAR logic circuit is conceptually straightforward, but it cannot be easily constructed since it requires accurate calibration of each receptor's signal intensity. For example, the iCAR may not block the signal if the aCAR signalling is too powerful.

Controlling the number of receptors on the cell surface is one of the most straightforward methods to alter the potency of CAR signalling. It is most convenient to vary the number of functional receptors on the cell using a split universal CAR arrangement. A universal receptor and an adapter protein that binds to the target cell and the universal receptor comprise a split CAR design.

The number of functional CARs and, hence, the signalling intensity may be adjusted by changing the adaptor protein concentration. In recent years, several split CAR designs have been developed [24–26].

Sophisticated, orthogonal leucine-zipper universal CAR receptors (zipCARs) and leucine-zipper "adaptor" domains that connect the zipCAR receptors to a range of antigens designated by a single chained variable fragment (scFv) domain (zipFv) are available in the SUPRA CAR system, which is the most adaptable system (*Fig. 2b*).

With zipFv titration and antigen-specific activation, the SUPRA CAR system demonstrated adjustable CAR activation. Leucine-zipper pairing orthogonality was used to show a range of logic operations (OR, AND, NOT) using zipFvs of different affinities against many antigens both in vitro and in vivo [18].

Despite having a high degree of modularity, SUPRA CAR is a more complex treatment that combines cell and protein therapy. Due to the protein nature of the adaptor molecule, zipFv may exhibit reduced permeability into the intended tissue, a shortened half-life, and maybe unidentified immunological responses. The context will probably determine which SUPRA CAR indication is acceptable.

2.5 | SynNotch

According to [27], [28]. the Lim group's synNotch receptor is a unique method for teaching CAR T cells to think logically. A programmable transcription factor that is directed against the promoter of the target gene is sandwiched between an external antigen-binding domain and the Notch receptor's proteolytic transmembrane core in a synNotch receptor.

According to *Fig. 2c*, transcription activation and the release of the transcription factor are the results of ligand binding to the synNotch receptor, which also cleaves the core Notch domain. An engineered surface ligand-induced mechanism for gene expression is the synNotch receptor.

Previously, the Lim lab used synNotch to create intricate tissue patterns and rewire immune cells [29]. Roybal and colleagues have designed a set of modular and humanized proteolytic-based receptors that bear similarities to the synNotch [16]. The majority of human components will reduce immunogenicity and make clinical translation easier. Using an IF-THEN logic, the synNotch-based logic circuit achieves AND or NOT logic, respectively, by causing the production of a CAR or an apoptotic gene upon synNotch activation [28], [30].

The CAR and the synNotch can each aim toward a distinct cage domain and can only be accessible to activate the CAR antigens when the cage and key are coupled to the same target cell, resulting in multi-input logic circuits. Even against glioblastoma, a solid tumour notorious for its great antigen heterogeneity, the AND logic performance of the synNotch-based circuit appears to provide enhanced specificity [31].

The antigens don't need to be on the same cell for a synNotch-based circuit to function. The antigen for the synNotch is no longer required when the CAR is produced. Consequently, if the healthy cells that are off-target and expressing the CAR antigen are near the targeted tumour cells, they may also be eliminated [32].

2.6 | Co-LOCKR

Split CAR architecture is also the foundation of the Co-LOCKR CAR system. According to *Fig. 2d*, the Co-LOCKR mechanism only uses one receptor. A group of computationally created adapter proteins that can interact with one another and change how the adaptor proteins attach to the CAR when target antigens are present enable the logic function.

The cage and key proteins, each of which has an antigen-binding domain, make up the core of the Co-LOCKR system. A peptide that can attach to and activate CAR T cells is also available in the cage protein. On the other hand, a latch domain isolates the cage's peptide domain.

The CAR is activated when the cage protein and key protein link together, causing a conformational shift that makes the peptide accessible for binding. Key proteins and the cage are not meant to interact in solution. On the contrary, once they are colocalized to the cell surface via antigen-binding domains, the equilibrium promotes the formation of cage-key complexes.

In CAR designs, co-LOCKR switches have been used to target up to three distinct antigens on cancer cells. Additionally, AND, OR, and even complex logic like A AND B NOT C may be used with this split CAR system [17].

The Co-LOCKR design depends on the presence of the key protein to unlock the cage rather than the balance of intracellular signaling domains. Nevertheless, using a decoy key protein to produce NOT logic has a drawback since the logic depends on the amount of the decoy protein.

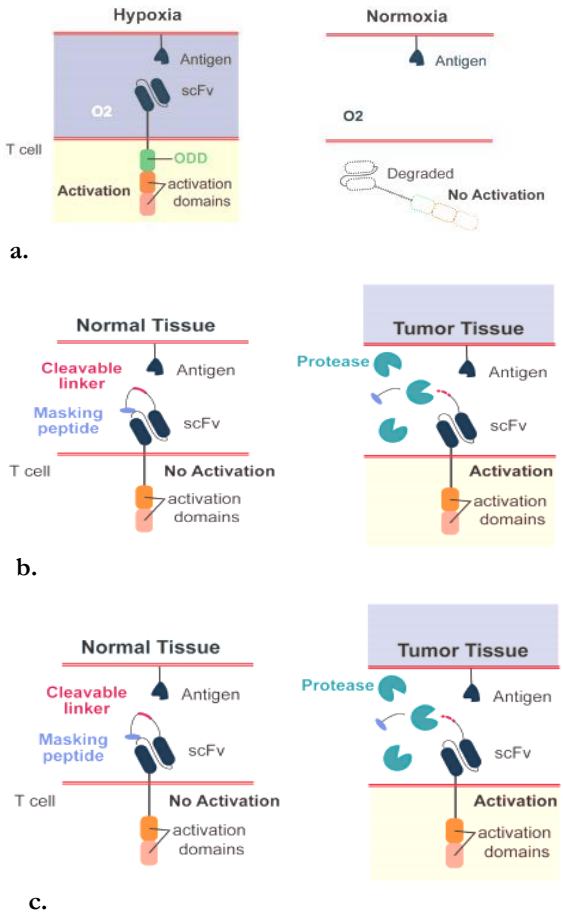

Fig. 3. System designs for cell-state-based control; a. Oxygen-based CAR control, b.Tumor-sepecific protease, and Activation-state.

We say in the explanation of *Figs. 3a-3c* a CAR whose stability is on hypoxia and has an Oxygen-Dependent Degradation (ODD) domain. Under normal oxygen concentration, the ODD will break down, which will cause the CAR to break down. A CAR architecture that requires tumour-specific protease to be present. The scFv is hidden by a masking peptide and a cleavable linker, which the protease can cleave off to reveal the scFv and initiate the CAR against the target antigen. The immune cell's activation state can be used to regulate the production of further cytokines. The target cytokine transcription is induced by the dephosphorylation and translocation of NFAT into the nuclease upon T-cell activation.

2.7 | Cell-State-Based Control

Not all signals can reroute the cytotoxicity of immune cells, including cell surface antigens. Due to the concentration of immunosuppressive metabolites and immune inhibitory molecules that restrict cytotoxic immune activity, the tumour microenvironment is frequently immunosuppressive [33], [34].

A potent tactic to combat the tumour microenvironment is to modify immune cells to detect certain characteristics of the microenvironment and to create substances that enhance anti-tumour activity. While cell state-based therapies could improve therapy specificity, there's a chance they could also result in less effective activity.

Once the T cell is activated, the NFAT is dephosphorylated and translocated into the nuclease to stimulate the target cytokine transcription. This is because shrinking tumours may also result in a decrease in representative cell states. Reducing the specificity or efficacy of the treatment for cell-state-based control approaches, striking a balance between specificity and activity would be essential. Oxygen-driven CAR

regulation Hypoxia, or low oxygen tension, is a defining feature of solid tumours and is frequently localized due to uneven vasculature and dense cell mass [35]. Thus, hypoxia may perform input signals to improve the specificity of CAR T cell therapy's tumour targeting.

Fusing an ODD domain to a CAR can result in a hypoxia-inducible CAR structure by making the CAR's stability hypoxic [36].

Fig. 3a in vitro, this ODD-fused CAR showed hypoxia-induced cancer cell death, although significant basal killing at ambient oxygen levels was also seen. Using a synthetic hypoxia-inducible promoter to regulate the transcription of ODDCAR, an alternate strategy that expands upon the ODD-fused CAR idea, offers two degrees of control over CAR activity. As a recognized issue with some CARs, such as antiHer2 [37], the HypoxiCAR T cell [38] can infiltrate tumours, resulting in partial tumour clearance without Cytokine Release Syndrome (CRS).

To ensure the safety of this hypoxia-regulatable CAR T cell treatment, more research on the HypoxiCAR's performance in hypoxic environments over an extended time in normal cells is required. Proteases specific to tumours are frequently secreted by tumours to aid in invasion and different phases of tumour growth. Tumor-specific proteases may, therefore, serve as a marker for cancer treatments and diagnostics.

A masked anti-EGFR CAR T was recently created by Han et al. [39] prefixing the scFv domain with a masking peptide that contains a proteolytic site (*Fig. 3b*). By default, the masking peptide prevents CAR activation by blocking the antigen-binding site. But when a tumour-specific protease is present, the masking peptide is broken down, revealing the scFv and facilitating antigen binding and CAR T cell activation. Despite surrounding target antigens in vitro, the masked CAR T cells exhibited decreased activity without proteases.

In a subcutaneous human lung cancer xenograft model, masked CAR T cells showed activity akin to unmasked CAR T cells, suggesting the breakdown of the masking peptide.

To bolster the safety of CAR T in the clinic, an examination of relevant animal studies' off-tumour activities will be conducted. The state of activation of cells' immunological modulatory elements, including cytokines, is critical for preserving immunological homeostasis and thwarting infection and malignancies. Therefore, research is being done on cytokines such as IL-2 and IL-12 as anti-cancer therapy.

Bell and Gottschalk [40], Hoyos et al. [41], and Liu et al [42] have investigated the use of cytokine injection in combination with treatment as a means of improving CAR performance.

According to several studies [43–48], systemic cytokine delivery can, however, have major adverse consequences. Therefore, to reduce systemic toxicity, it would be ideal for the CAR T cells to generate the cytokines in the tumour microenvironment.

Making cytokine production conditional on CAR activation is one way to guarantee localized production. The cytokine production in CAR T cells was regulated by the nuclear factor of activated T cells (NFATs)/IL-2 composite promoter, which has been utilized for a considerable amount of time as a reporter of T cell activation [49–51]. (*Fig. 3c*).

The CAR T cells have been shown to function as a cytokine factory thanks to the exploration of IL-12, IL-18, and IL-21 [52–56].

2.8 | Exogenous Gene Control Circuits for Therapeutic Immune Cells

Enhancing the safety of the modified immune cells by restricting T cell activity in the case of unfavourable side effects or improving tumour-targeting selectivity are two of the most significant objectives of exogenous gene control circuits. As a result, two crucial factors in the design of the inducible system are the inducer's pharmacokinetics and safety profile.

Clinically speaking, there are several advantages to using a safe, authorized inducer, and this helps new CARs with improved safety profiles reach the market. Using an inducible switch also has the added benefit of greater durability and safety.

According to [57] drug-gated CARs can temporarily halt tonic receptor signalling, allowing T cells to recover from fatigue and eventually increase their in vivo persistence and anti-tumor effectiveness. According to the kind of inducer, there are now three groups of exogenous gene control circuits: light, ultrasound, and tiny chemicals. Each system functions as an ON or OFF switch when incorporated into immune cells, and external inducers control this response variation.

Except for recombinase-based or kill-switch systems, the majority of these systems lack memory. As a result, to keep the ON or OFF state, the inducer must always be present. As a result, the inducer's toxicity and mode of administration are crucial.

Furthermore, the characteristics of the system's parts determine whether an ON or OFF switch should be included. However, it is still unclear if having an ON or OFF switch is better from a clinical standpoint. We propose that an ON switch, needing continuous induction, is most appropriate when the output it regulates is potentially hazardous at high concentrations (a pleiotropic cytokine or an overactive CAR), necessitating meticulous management and fine-tuning.

On the other hand, an ON switch has the advantage that it can be turned off by simply withdrawing the inducer when the output is no longer needed; an OFF switch, which stays ON without any inducer, is best used with a relatively safe output (a well-behaved CAR) and only needs to be turned OFF in case of severe side effects.

2.9 | Small Molecules

Controlling the CAR directly is the easiest method to produce drug-gated CAR T cells. Drug-gated control is commonly achieved by inducible receptor stabilization or assembly. Usually, the CAR is divided into domains for antigen recognition and signalling as part of the assembly mechanism. To help (ON switch) or interfere (OFF switch) with the component assembly, a small molecule is employed [58–61]. A tiny molecule called degron, which has a controlled degradation domain, is fused to the CAR as part of the stabilizing process.

The CAR can be unfolded or cleaved by these degrons, and the inducer's binding can either stabilize the degron or prevent proteolysis (*Fig. 4a*, ON switch). When the small molecule inducer (OFF switch, *Fig. 4b*) is present, some degrons will activate their proteolysis machinery.

Lately, non-structure 3 (NS3) protease from the Hepatitis C Virus (HCV) has been used to create inducible CAR systems [58], [61], [62]. One benefit of the NS3 system is that clinically authorized protease inhibitors with a good safety record, may control it.

Typically, an inducible system can only use the stabilizing mechanism or the assembly. On the other hand, certain systems, like the lenalidomide system [58], [59], [62] or the Versatile Protease Regulatable CAR (VIPER CAR), may use both processes to construct ON and OFF switches using the same inducer. Moreover, Li et al. [76] have demonstrated that multiplexed control circuits that might enhance the safety and specificity of CAR T cell treatment can be created by combining the NS3-based system with other CAR designs, such as SUPRA or the lenalidomide system.

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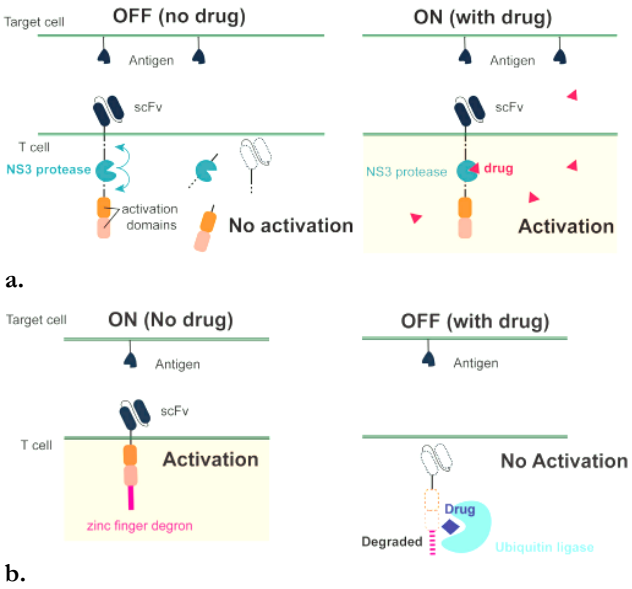

Fig. 4. Exogenous cell control with ON and OFF switches; a. ON-switch, and b. OFF- switch.

Fig. 1a shows an NS3 protease is used in this example ON switch to regulate the CAR activity. Only in the presence of a medication that inhibits protease action can CAR be stable, allowing for signal transmission.

Also in *Fig. 2b* An example of an off switch that uses a synthetic ubiquitin ligase and the zinc-finger degron motif. A medication that can cause the degron and the ligase to dimerize will alert the CAR to degradation, preventing the drug from activating T cells.

A safer solution to lessen off-target effects would be programmable synthetic transcription factors, including those based on CRISPR or zinc finger. For example, the synthetic zinc finger transcription regulators, or synZiFTRs, were engineered to lie perpendicular to the human DNA. To control the expression of CAR and cytokines in human primary T cells, many inducible synZiFTR systems have been created utilizing clinically authorized medications as the inducer [62]. This is the first dual inducible gene expression control system of its kind.

Natural products, such as resveratrol, present in red wine, grapes, and berries, have also been used to repress or induce CAR expression in addition to clinically approved drugs. This has demonstrated the applicability of CAR expression in primary T cells with a high dynamic range, both in vitro and in vivo [63].

It will affect gene expression over the long term with brief drug exposure thanks to induced gene switches with memory functions. This function will reduce the requirement to provide the medication inducer continually, which might be helpful in situations where giving the drug continuously could be harmful or difficult.

Drug-inducible CAR expression with memory was created to induce CAR expression via a recombinase-based gene circuit with the FlpO-ERT2 fusion protein [64].

One may utilize the circuit to switch on or off CAR expression, depending on how the target gene was originally designed. Immune cells have been engineered to produce photoactivable CARs using light-inducible dimerization domains (*Fig. 5a*) [65]. Prior research has shown that a Blue-light-inducible system with a localized CAR expression system in T cells [66], [67].

Zhao et al. [68] employed an analogous optogenetic method to stimulate T-cell cytokine production to eradicate cancer cells. Exact spatiotemporal control with negligible side effects can be achieved with a noninvasive light-inducible system, which is challenging to do with a small molecule-inducible gene expression system. However, the therapeutic uses of blue light are limited due to its shallow tissue penetration depth (less than 1 mm). A nanoplate technique that can upconvert Near-Infrared Right (NIR), a more

transmissible light in tissue, into blue light has been created to overcome this constraint. Reversible and real-time regulation of the CAR activation was achieved to reduce the possible cytokine storm by injecting the nanoplate with blue light-inducible CAR T cells into tumour-bearing mice [69].

2.10 | Ultrasound

Because ultrasound is safer and has a deeper penetration than light, it makes a compelling physical inducer, given the difficulties associated with utilizing light. Pan et al. used the ultrasonically triggered Piezo1 calcium channel, which is mechanically sensitive [70]. Ultrasound exposure produces microbubbles, which open the Piezo1 channel and allow calcium entry into the cell. When calcineurin is activated by a calcium influx, an NFAT transcription factor is subsequently dephosphorylated.

Following ultrasonic exposure, CAR transcription was induced using the NFAT-responsive promoter. However, the need for microbubbles prevents them from being used in vivo. The same team of researchers created a heat-induced CAR that reacts to ultrasound to get around this problem [71]. Heat shock protein promoter expressing Cre recombinase can start and sustain CAR expression, and focused ultrasonic waves raise the local temperature (*Fig. 5b*). To regulate T-cell activity, Miller et al. used a heat-responsive component [72].

Rather than initiating CAR activity directly, they converted NIR into heat using a plasmonic gold nanorod. When T cells express CAR, this technique successfully demonstrates T cell trafficking to the antigen-expressing tumour. In addition, they employed a plasmonic gold nanorod to generate heat from NIR. They showed that the heat produced might stimulate the production of bispecific T cell engager (BiTE) to prevent tumour expansion caused by antigen escape and IL-15 superagonist to increase CAR activity in vivo.

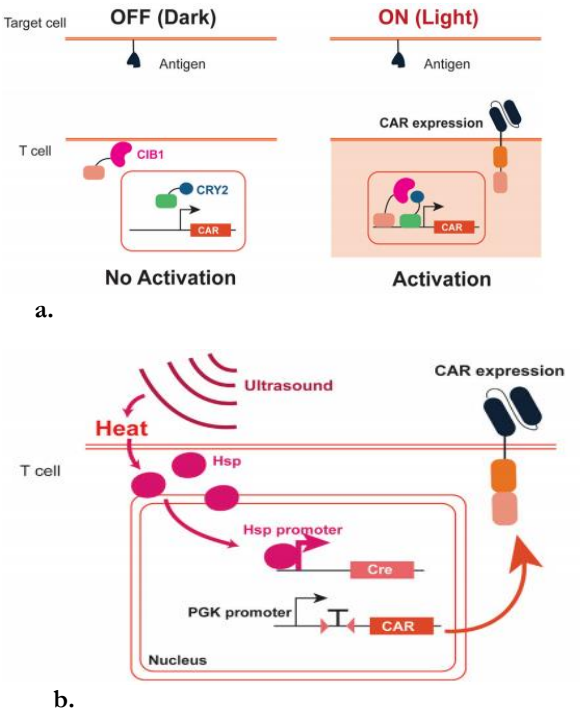

Fig. 5. Exogenous cell control with light and ultrasound inputs.

Fig. 5a explains that two light-inducible dimerization domains coupled to transcription regulatory elements make up the design of the photoactivable CAR activation system. The T cells will activate the CAR upon light induction, and *Fig. 5b* Heat Shock Protein (Hsp)-mediated gene expression is used in the ultrasound-inducible CAR system.

3 | Discussion

The fact that cells can perceive their surroundings and carry out different duties makes employing them as treatments one of the most fascinating aspects of modern medicine. Therefore, it would be ideal to devise a plan for incorporating a variety of characteristics and functions into cell treatments while simultaneously attending to safety, specificity, and effectiveness issues. As was already said, several effective techniques have been created to address these difficulties. We presented the cell-autonomous and cell-exogenous control arms of immune cell switches. The two arms can be used in tandem and are not exclusive. Overall, CAR T cell therapies will be improved even more by our capacity to rewire receptor machinery and create new methods of controlling the engineered immune cells.

Heat-producing ultrasound raises the local temperature and triggers Hsp translocation. Cre expression is induced by Hsp translocation, and it is Cre that mediates the expression of CAR on the T cell. Some of the concepts mentioned here are more applicable to the clinics than others, even if many are still in their early stages. Logic CARs appear to be the most popular right now. For example, 2-input OR gate CARs have previously undergone clinical evaluation and demonstrated encouraging outcomes [73]. NIMPLY (A AND NOT B) gate CARs are being actively pursued by several businesses for a variety of tumours [23], [74].

To increase the anti-tumour efficacy, several CAR T cell treatments have also been developed to create factors, such as prodrug modifying enzymes [75] immunomodulatory factors [40], [76] or checkpoint inhibitors [77]. Although necessary, such designs also increase the possibility of serious negative side effects. Thus, to strike a balance between activity and safety, regulatable control of CAR activity and transgene expression will be required. Given their ability to offer the necessary safety control, several of the drug-inducible CARs and gene switches discussed here, particularly those utilizing pharmaceuticals that have received clinical approval, are probably going to enter clinics very soon.

4 | Conclusion

One of the main obstacles preventing science from reaching its full potential is the capacity to genetically modify human immune cells on a massive scale, particularly those produced from primary sources. Despite the progress made in cutting-edge genome editing techniques using CRISPR systems [78], a significant obstacle that is difficult to overcome is the integration and delivery of huge DNA payloads. Creating a group of immune cells that independently carry the cell-autonomous and cell-exogenous systems like the human immune system is one way to get around this problem.

According to [79] it may be possible to transfer these smaller genetic programs to T cells in situ, avoiding the requirement for the labour-intensive ex vivo manufacturing procedure and reducing the therapy's cost. Our ideal scenario involves delivering complex genetic circuits with improved safety characteristics, effectiveness, and specificity in situ into various types of immune cells, enhancing the patient's immune system to fight and prevent a wide range of illnesses.

Author Contributions

Daniel Oluwatobi AKINTAYO conceptualized the study and led the research on recent improvements in gene regulations and immunology. Babatunde Ridwan SALAM contributed to analyzing cell-autonomous versus exogenous control, design considerations, and cell-autonomous circuits for therapeutic immune cells. Adetayo Olaniyi Adeniran assisted with the overall coordination and writing of the manuscript. All authors contributed to the literature review and approved the final manuscript.

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Data Availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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