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Synthetic Biology Approaches for Cancer Immunotherapy Innovation

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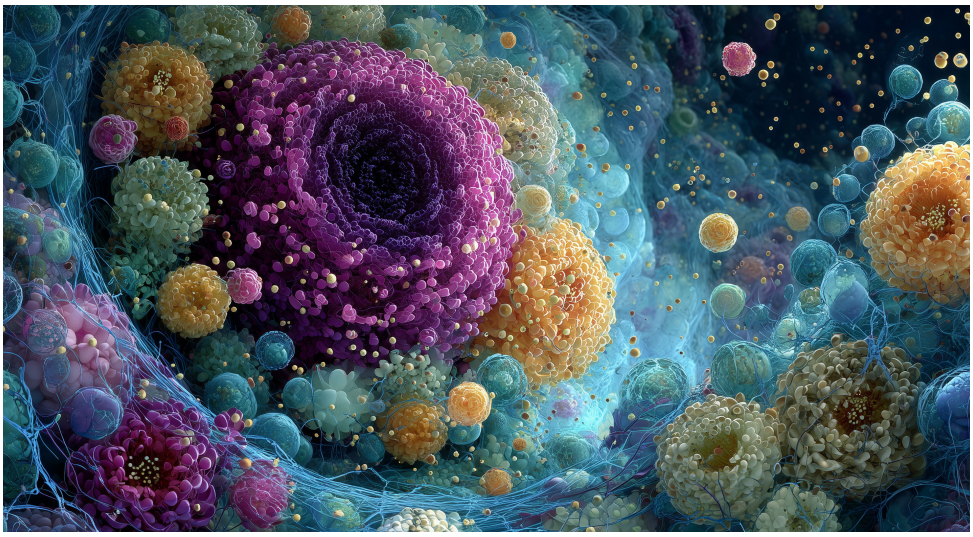

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Abstract

Immunotherapies have had a significant impact on efficacy of cancer therapies and resulting survival rates and are relatively efficacious treatment options for a wide variety of cancer types. However, there are many challenges that remain in the development of safe and long-lasting efficacious cancer immunotherapies. Synthetic biology approaches have the potential to improve the current cancer immunotherapies used in the clinic in terms of toxicity, specificity, tunability, and efficacy. In this way, engineered cells, genes, and vaccines can be utilized to develop novel immunotherapeutic strategies for cancer patients. However, it remains important to consider ethical concerns that arise with genetic modification, as well as economic and regulatory challenges that restrict clinical translation and accessibility. This review article provides an overview of innovative cancer immunotherapies that utilize common synthetic biology-based approaches to target tumor cells through leveraging the body's own immune system.

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1. Introduction

Cancer is among the leading causes of death worldwide. In 2022, there were almost 20 million new cases and 9.7 million cancer-related deaths globally.[1] It is estimated that by 2040, the number of new cancer cases per year will rise to 29.9 million and the number of cancer-related deaths to 15.3 million.[1] Thus, it is apparent that existing FDA-approved cancer therapeutics are not sufficient, and there is a need to produce more efficacious cancer treatments that are safe for patients.

Cancer immunotherapies are treatments that leverage the body's own immune system to recognize and attack cancer cells. They have been able to revolutionize the field, transforming the landscape of cancer treatment due to their advantages over some conventional treatment approaches. Currently, there are a variety of FDA-approved immunotherapeutic options and many more in clinical trials. However, although immunotherapies are commonly prescribed by physicians as both monotherapies and combination therapies for cancer treatment, only 20-40% of patients respond to these immunotherapeutic strategies.[2,3] This is due to the various challenges that continue to impede further immunotherapy development, including on-target off-tumor toxicity, systemic toxicity, and the difficulty of achieving long-lasting therapeutic efficacy.[4] Because these drugs can activate a broad range of immune cells, they can also sometimes trigger severe auto-immune reactions. Thus, these concerns need to be addressed in order to improve these types of therapies, and synthetic biology-based approaches have the potential to advance the field through the development of specific and controllable cell therapies, gene circuits, and vaccines.

Synthetic biology involves engineering systems with various innovative tools to implement customized functions into existing living systems. Thus, this multidisciplinary field of science utilizes engineering principles, like modular design, standardized parts, and systemic simulation to develop new or redesign existing biological systems.[4] Synthetic biology has specifically been applied in the engineering of cell and gene therapies, microbes, programmable genetic circuits, components for vaccine production.[4] In this way, synthetic biology methods offer promising solutions to the obstacles presented by existing cancer immunotherapies, allowing for innovation in

terms of safety and efficacy. Specifically, synthetic biology, through the development of engineered cells, gene circuits, and vaccines, has the potential to revolutionize targeted and programmable cancer immunotherapies.

2. Cancer Immunotherapies

There are a wide variety of clinically available cancer immunotherapies that can be selected for specific patients depending on the cancer type, severity, and other medications or therapeutics being provided. Some examples of clinically available cancer immunotherapies include immune checkpoint inhibitors, monoclonal antibodies, cytokine therapies, adoptive cell therapies, and cancer vaccines.

Immune checkpoint inhibitors block checkpoint proteins on immune cells, allowing them to attack cancer cells more effectively. In preventing checkpoint from binding to their partner proteins, T cells are able to kill tumor cells.[5] Antibodies targeting immune inhibitory receptors, such as CTLA-4, PD-1, and PD-L1, have been the most widely used immunotherapeutic agents in the last decade.[5] Specifically, PD-L1 on tumor cells and PD-1 on T cells bind, preventing T cells from killing tumor cells within the body. However, blocking this binding with either anti-PD-L1 or anti-PD-1 will allow the immune system to recognize and destroy the cancer cells. Immune checkpoint inhibitors have been approved to treat a variety of solid tumor cancer types, as well as some lymphomas, but can cause a variety of side effects, including rash, diarrhea, and fatigue.[6]

Monoclonal antibodies are antibodies made by clones of a unique B cell, all of which bind to a specific epitope. Antibodies have the ability to both directly kill tumor cells and develop long-lasting effector responses against the cancerous cells by engaging with the patient's immune system.[7] They can help the immune system to recognize and attack tumor cells, as monoclonal antibodies are an example of personalized therapeutics based on specific disease characteristics.[8] Targeting tumor-specific antigens with monoclonal antibodies can result in tumor cell death through a variety of mechanisms. Monoclonal antibodies are laboratory-produced to mimic the body's natural antibodies, allowing them to block growth signals, mark cancer cells, or deliver cytotoxic drugs without an immunogenic response.[7] The target

specificity of monoclonal antibody therapy results in strong anti-tumor responses, while minimizing toxicity and adverse systemic effects.[7] Thus, there are many FDA-approved monoclonal antibody therapies for various solid and hematological cancer types.

Cytokines are proteins that play a major role in the regulation of the innate and adaptive immune systems. Cytokine therapies leverage these signaling molecules to stimulate the growth and activity of immune cells that can recognize and destroy cancer cells.[9] Particularly, interferon-α (IFN-α) and interleukin-2 (IL-2) are cytokines that have been approved for the treatment of specific human cancers.[10] Cytokine therapies generally need to be administered in large quantities in order to achieve necessary concentrations within the tumor, resulting in toxicities and severe side effects.[9] These cytokine therapies are also frequently used in combination with other immunotherapies to enhance efficacy.

Adoptive cell therapies involve using the patient's own immune cells to target cancer cells, and some common examples include chimeric antigen receptor (CAR)-T cell therapies and engineered T cell receptor (TCR)-T cell therapies.[11] Adoptive cell therapies employ the patient's own immune cells to stimulate antitumor immunity.

Cancer vaccines involve presenting tumor antigens to train the immune system to recognize and destroy cancer cells. Some examples include dendritic cell, peptide, and nucleic acid-based vaccines.[12] Cancer vaccines may introduce tumor-specific antigens, whole cancer cells, dendritic cells, or nucleic acids from cancer cells in order to prompt the immune system to activate immune cells and produce antibodies that can destroy these tumor cells.[12] These conventional cancer immunotherapies have been engineered using synthetic biology-based methods to improve their performance. Thus, this review will highlight the effect of synthetic biology approaches on cell-based cancer immunotherapies.

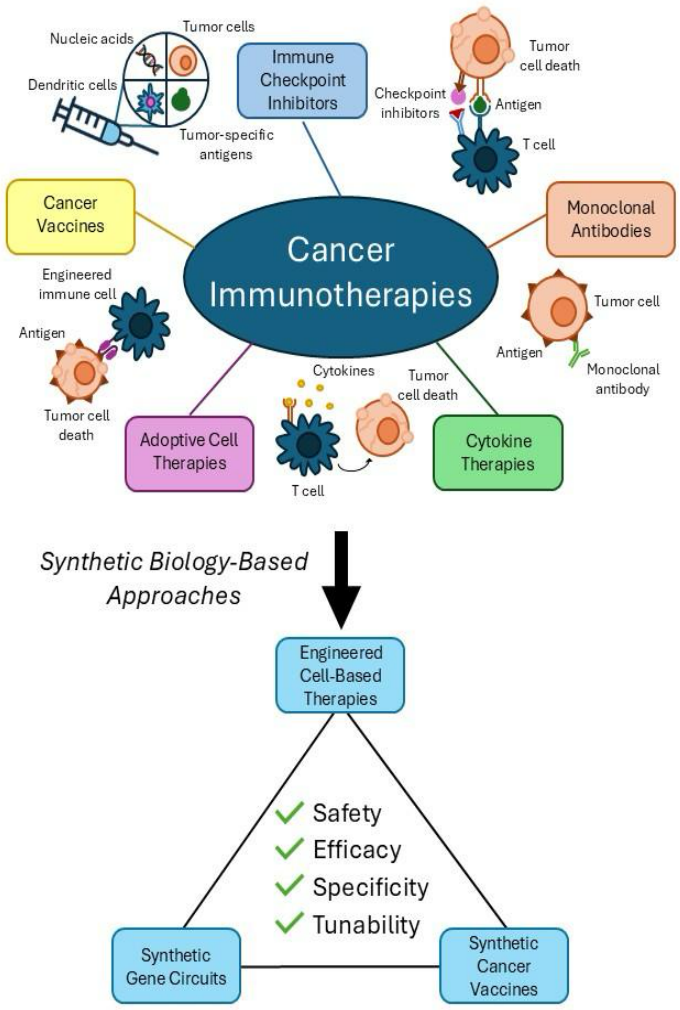

Figure 1: Graphical abstract detailing types of cancer immunotherapies and advantages of synthetic biology-based approaches.

The types of cancer immunotherapies with FDA-approved treatments that are currently used clinically include immune checkpoint inhibitors, monoclonal antibodies, cytokine therapies, adoptive cell therapies, and cancer vaccines. Through the use of synthetic biology, engineered cell-based therapies, synthetic gene circuits, and synthetic cancer vaccines have the potential to revolutionize cancer immunotherapies in terms of toxicity, specificity, and programmability., improving the safety and efficacy of these treatments.

3. Novel Synthetic Biology-Based Cancer Immunotherapies

3.1 Engineered Cell-Based Immunotherapies

Immune cells are being engineered and designed to target certain diseases and pathogens to improve efficacy and accessibility of treatment. There are several types of cell-based treatments that have risen from advances in cell engineering.

CAR-T cells, one of the well-known forms of engineered cells used against cancers, provide for important cell-based therapies for cancers. There is vast research done and is being done on the efficacy of CAR-T cell therapies for different types of cancers. Notably, the development of CAR-T cells has been at the forefront of immunology and oncology research for years. There are already six FDA-approved chimeric antigen receptor modified T cell therapies for the treatment of hematological malignancies.[13] Unlike other forms of therapies aimed at cancer, such as protein biologics, radiation, or molecular drugs, cell-focused immunotherapies are living interventions that can actually travel throughout the patient's body.[13] Such immune cells carry out important immune responses that target tumor cells and prevent metastasis of cancers. T cells require a major histocompatibility complex to activate it towards a specific antigen.[13] T cells are being engineered with chimeric antigen receptors, which allow for the immune cells to be activated without needing a major histocompatibility complex.[13] The different CAR-T cell therapies involve the CAR-T cells being designed to detect specific antigens to target cells affected by a specific disease, like cancer. There are several clinical trials that show CAR-T cell therapies were tolerated well and had promising efficacy in various types of cancers, primarily blood cancers.[13] Clinical trials have shown that engineered CAR-T cells have given a template to construct more targeted and more potent immune cell therapies for cancer.[14] In another study that examines its use in multiple myeloma, SLAMF7 CAR-T cells show consistent anti-myeloma activity in both in vitro and in vivo in the preclinical phase.[15] CAR-T cell therapies show strong potential in various types of malignancies, particularly in hematological malignancies. A trial done in China studied the effectiveness of administering allogeneic anti-CD19-CAR-T cells to patients with B-cell malignancies, and the trial suggested that the therapy expanded and induced

durable remission in patients with manageable safety profiles.[16] To further expand on CAR-T cell therapy's promise against cancers, a clinical study was done of castration-resistant, prostate cancer-directed CAR-T cells with a dominant-negative TGF-B receptor, which is an inhibitory factor in the immunosuppressive tumor microenvironment. There was a 98% reduction in prostate-specific antigen and acute increases in inflammatory cytokines in these patients.[17] Research also shows that thoracic cancers, which refer to malignancies in the thoracic cavity, are potential targets for the use of CAR-T cell therapies.[18]

Further, NK cells are part of the innate immune system and have a shorter life span with a smaller potential to proliferate. They do not have receptors that recognize a broad spectrum of antigens, but they do activate cytotoxicity when a ligand is bound to their germline-encoded receptors.[14] Essentially, NK cells are activated by more general stress signals from cells, leading them to be toxic to such infected cells. Clinical trials have shown that NK cells have modest outcomes when targeting tumor cells, but with more advances in engineering CAR-T cells have given a template to construct more targeted and potent NK cell therapies for cancer; the use of chimeric antigen receptors (CAR) for NK cells have shown the ability to control NK cell cytotoxicity in a more antigen specific manner.[14] There are still various challenges that are yet to be overcome for the current cell therapies to be viable. Notably, these obstacles are selecting an appropriate target antigen and combating immunosuppressive cells and signaling molecules present in the tumor microenvironment. There are certain stem cells, called induced pluripotent stem cells (iPSCs) that have immense potential as a source for both NK and T cell based therapies because of their unlimited proliferation potential.[18] CAR-NK cell clinical trials have shown promise in the field of cellular therapy, but further research needs to be done in order for the therapies to have broader therapeutic implications.

TCR-T cells are another promising immunotherapy used against cancer. TCR-T cells are another form of engineered T-cells. These cells are also modified to recognize and bind to specific antigens.[19] These cells are primarily used against tumors and cancers; consequently, TCR-T cells are engineered to specifically recognize different types of cancers. Unlike CAR-

T cells, TCR-T cells are major histocompatibility complex dependent, meaning that they require a major histocompatibility complex molecule to present antigens to the surface of TCR-T cells in order for them to be activated.[19]

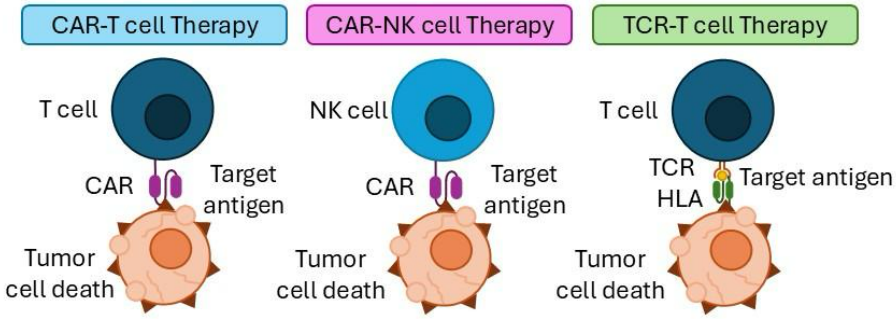

Figure 2: Engineered adoptive cell therapies

CAR-T, CAR-NK, and TCR-T cell therapies are examples of adoptive cell therapies that utilize synthetic biology to genetically modify immune cells to express specific receptors, allowing them to recognize and bind to target antigens on cancer cells.

To further advance the field, hematopoietic stem cell gene engineering and in vitro differentiation is combined to generate human allogeneic HSC-engineered invariant natural killer T cells. These cells can target tumor cells while having high safety and low immunogenicity. HSC-INKT cells were observed to express higher levels of NK activating receptors and confirmed its antitumor potential.[19] HSC-iNKT cells can be used as the allogeneic cell carriers for CAR-directed cell therapy. They have high antitumor potential because of their ability to co-express memory T cell and NK cell markers.

The use of pluripotent stem cells is prevalent in treating several diseases today and remains an area of study with strong potential and implications. Embryonic stem cells, for instance, were clinically tested in proliferating a retinal pigment epithelium (RPM) for patients with age-related macular degeneration, which showed optimistic results.[20] Even patients with blood cancers are treated with hematopoietic stem cell transplantation. In this process, stem cells derived from the patient or a donor's bone marrow are placed in the system of the patient with blood cancer. These stem cells are

involved in attacking cancerous blood cells and can restore stem cells in the blood that may have been damaged after chemotherapy.[18] Stem cells are an important aspect of cell based therapies as many engineered cells can be derived from pluripotent stem cells. Because of their immense proliferation potential, these stem cells are a source for NK and T cell based therapies for tumors.

Engineered cell-based therapies such as CAR-T cells and TCR-T cells have demonstrated tremendous potential in targeting a wide range of cancers. However, while these therapies leverage synthetic biology for receptor engineering, they often lack the precision and dynamic control needed to adapt to complex tumor environments. This is where synthetic gene circuits come into play. By integrating programmable logic gates, dynamic response modules, and environmental sensing into immune cells, gene circuits allow for precise, context-dependent control of immune activity. The following section explores the design and application of synthetic gene circuits in cancer immunotherapy, highlighting recent advancements that have redefined the capabilities of engineered immune cells.

The advancement of adoptive immune cell therapies has established a strong framework for targeted cancer immunotherapy. These modalities have presented significant promise in the clinical and preclinical stages. There are still various challenges that must be overcome in precision, adaptability, and tumor microenvironment resistance.

3.2 Synthetic Gene Circuits

Engineered immune cells with programmable genetic circuits are being developed to overcome the limitations of conventional therapies.[21] Synthetic biology enables design of “logic” modules and inducible systems that can precisely control immune cell behavior in the tumor microenvironment. For example, CAR-T cells targeting CD19 have been transformative in leukemia, but strategies are needed to address solid tumors, antigen escape, and off-target effects.[21] Synthetic circuits promise to provide that control, implementing Boolean logic and environmental sensing to improve specificity, safety, and efficacy.[21,22] The circuit can be used in cancer immunotherapy, focusing on T-cell functionality, dynamic immunomodulation, innate cell engineering, and CRISPR-based networks.

Synthetic receptor circuits are widely used to augment T-cell targeting and activation. Engineered receptors (e.g. CARs, synNotch, SUPRA CARs) can be combined in logical configurations so that T cells respond only to defined antigen combinations or contexts. By functioning as molecular logic gates, synNotch receptors enable precise multi-antigen regulation of T-cell activation.[22] For instance, synNotch can induce expression of a CAR or cytokine only when its cognate antigen is present, implementing an "AND" gate. Figure 3 illustrates modular designs such as SUPRA CARs (leucine zipper "zipCAR" + interchangeable "zipFv" ligand) and dual-antigen circuits (Co-LOCKR, series/parallel synNotch) that improve specificity.[22,23]

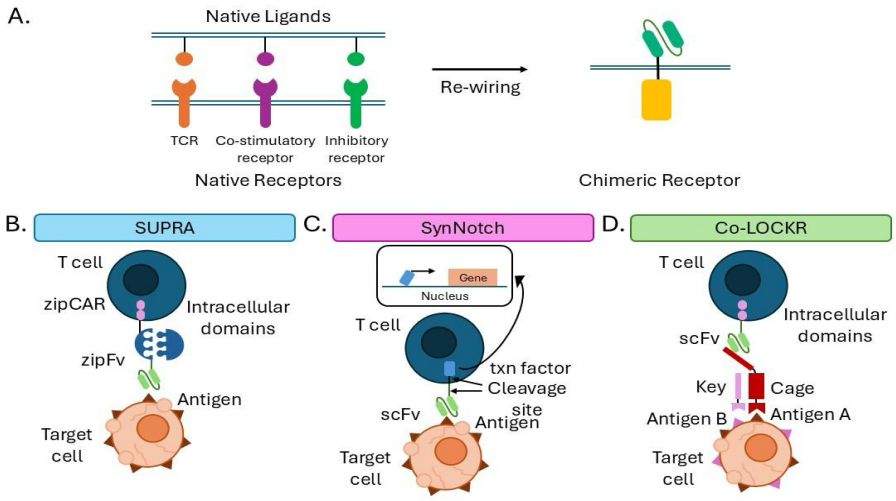

Figure 3: Engineered T-cell receptor circuits.

(A) Native immune receptors (TCR, costimulatory or inhibitory) can be rewired by chimeric fusion. (B) SUPRA CAR splits targeting (zipFv) and activation (zipCAR) domains for modular antigen binding. (C) SynNotch receptors release a transcription factor (txn) upon antigen binding, triggering custom gene expression. (D) Co-LOCKR "cage/key" system implements an AND-gate requiring two antigens for CAR activation.[23] Such designs aim to minimize off-target killing and adapt to tumor heterogeneity.

Advanced receptor platforms have recently extended these capabilities. Wang *et al.* developed a SNAP-CAR/synNotch system for post-translational retargeting: a SNAP-tag fused to the extracellular domain of a CAR or synNotch can covalently bind benzylguanine (BG)-conjugated antibodies,

effectively reprogramming T-cell specificity at the protein level.[24] In mouse models, SNAP-CAR T cells armed with BG-antibody adaptors were able to recognize and eliminate tumor cells in vivo, demonstrating a flexible "universal" targeting strategy.[24] Other logic circuits have been demonstrated by domain swaps or dual-receptor cascades. For example, dual-synNotch architectures can operate in series or parallel to sequentially activate an anti-tumor CAR only upon detection of primary and secondary antigens.[23] These logic-gated circuits are designed to "gate" T-cell activation to tumor-specific patterns, reducing collateral damage to normal tissues. Overall, recent studies show that modular receptor circuits significantly enhance T-cell precision and potency.[23,24]

Synthetic circuits also enable controlled cytokine release and time-dependent behavior. One key advance was the creation of autocrine cytokine circuits that locally boost T-cell activity. Allen *et al.* engineered CAR-T cells with a tumor-specific synNotch receptor driving IL-2 expression: the synthetic synNotch→IL-2 circuit produces IL-2 only upon encountering the target antigen.[25] This design bypasses tumor suppression (such as IL-2 scavenging or TCR inhibition) by providing a self-sufficient growth signal. The IL-2 circuit markedly improved CAR-T infiltration into immune-excluded tumors and tumor clearance in mice, without systemic toxicity.[25] In vitro and in vivo assays confirmed that synNotch-triggered IL-2 caused robust T-cell proliferation (autocrine and paracrine) only in the presence of tumor antigen, effectively "priming" the immune response.[25]

External physical triggers have also been demonstrated: in one study, activated ultrasound or light was used to induce CAR expression selectively in tumors.[25] More recently, Xue *et al.* engineered a heat-inducible switch in macrophages: a wearable thermal device (remote-controlled warming pad) mildly heats the tumor region, activating a CRISPR/dCas9-based circuit that induces IFN-γ production only in engineered macrophages.[26] This locoregional cytokine delivers polarized tumor-associated macrophages to an M1-like (proinflammatory) state, improving antitumor immunity without systemic inflammation.[26] Such circuits exemplify how user-controlled stimuli can dynamically reprogram immune function in vivo.

Beyond T cells, synthetic circuits are being applied to innate immune cells to remodel the tumor milieu. Key strategies include programming macrophages to deliver cytokines or antigens and converting cells into professional antigen presenters.

Synthetic circuits can polarize macrophages toward a tumoricidal phenotype on-demand. For instance, Xue *et al.* (2024) developed a CRISPR/dCas9 circuit in induced macrophages that is activated by mild hyperthermia.[26] When heated (via a wearable device), the circuit drives endogenous IFN-γ expression, triggering both engineered and resident macrophages to adopt a classically-activated (M1) state. This resulted in strong local inflammation and tumor growth inhibition with minimized systemic cytokine exposure.[26] In other work, researchers have designed NF-κB-responsive loops or cytokine-sensing promoters to make macrophages secrete inhibitors (e.g. soluble TNF receptors) in response to inflammatory cues, demonstrating the feasibility of autoregulatory anti-tumor drug delivery (though in non-cancer models).[26,27] Collectively, these approaches show that macrophage-based circuits can home to tumors and locally modulate the microenvironment.

Synthetic reprogramming can also convert cells into antigen-presenting cells to boost immunity. Zimmermannova *et al.* (2023) showed that enforcing expression of the minimal cDC1 transcription factors (PU.1, IRF8, BATF3) in tumor cells induces a DC-like phenotype.[28] The reprogrammed tumor-APCs expressed high levels of MHC and costimulatory molecules, secreted inflammatory cytokines, and effectively cross-presented tumor antigens to $CD8^+$ T cells.[28] In mouse models, injecting these engineered tumor-APCs into tumors reduced tumor growth and synergized with checkpoint blockade. This strategy "endows" cancer cells with DC functionality, effectively broadening antigen presentation within the tumor. In summary, recent studies use gene circuits to co-opt macrophages and DCs for cancer therapy, either by turning macrophages proinflammatory or by making cells better at priming T cells.[26,28]

CRISPR technologies are increasingly integrated into synthetic circuit design for immunotherapy. Engineered dCas9 proteins fused to activators or repressors can serve as programmable sensors and logic elements. For example, Wang *et al.* constructed a tumor-specific AND-NOT CRISPRa

circuit in human cells.[29] In this design, an oncogenic transcription factor activates a dCas9-VPR effector to drive immunostimulatory genes, while a p53-inducible "off-switch" (either an inhibitory sgRNA or an anti-CRISPR protein) serves as a NOT gate.[29] Only in cells lacking p53 (a common cancer trait) does the CRISPRa module remain active. This circuit showed precise tumor-cell recognition and induced potent antitumor immunity in vivo, demonstrating how CRISPR components can implement multi-input logic for targeted therapy.[29]

CRISPR circuits have also been used as on-demand actuators in immune cells. In the Xue macrophage study, the heat-inducible system was built by combining dCas9–GCN4 scaffolds with a p65-HSF1 activator to transcriptionally target the endogenous *Ifng* locus.[26] This highlights the flexibility of CRISPR/dCas9: by simply changing guide RNAs or effector domains, one can reprogram which cytokines or receptors are controlled. While CRISPR-based designs enable highly customizable regulation of cell states, challenges remain (e.g. guide RNA specificity, payload delivery). Nevertheless, recent work illustrates their promise: programmable CRISPR circuits can be wired into T cells or macrophages to sense oncogenic signals and precisely control anti-tumor outputs.[26,29] Continued advances in CRISPR engineering are expected to broaden this toolkit (for example, CRISPRi/CRISPRa libraries for pathway rewiring or epigenetic circuits), making CRISPR an integral part of next-generation immunotherapy platforms.

In summary, synthetic gene circuits are enabling next-generation immunotherapies that go beyond static receptor designs. By encoding complex logic, feedback loops, and sensory switches, these engineered circuits empower immune cells to make context-dependent decisions. Together, these strategies promise to enhance efficacy and safety by autonomously tuning immune function in time and space, heralding precision cell therapies tailored to the tumor environment. Such programmable and precise control over cellular behavior is not limited to immune cells—these same principles are being extended to other platforms, including the design of synthetic cancer vaccines, which leverage similar concepts to enhance immune targeting and durability.

3.3 Synthetic Cancer Vaccines

Synthetic vaccines can provide quality alternatives to traditional vaccines in cancer treatment with its precise editing, fast production, and safety. In synthetic vaccines, different components such as antigen structure or genetic material are engineered in the lab to induce an immune response.[30] Unlike traditional vaccines, they do not carry live pathogens, which mitigates the risk of disease for recipients.[31] Through recent research, they have been shown to slow down metastasis and tumor recurrence against cancerous masses. In particular, peptide-based, DNA, and mRNA synthetic vaccines have shown growing potential to become primary treatments for cancer immunotherapy in the future.

Features	Synthetic Peptide Vaccines	Synthetic DNA Vaccines	Synthetic mRNA Vaccines	Traditional Vaccines
Components	Amino acids holding selected epitope of antigen[32]	DNA plasmids with gene of interest[33]	In vitro transcribed mRNA[34]	Live / inactivated microbes
Method of Production	Solid phase synthesis or fragment condensation techniques[35]	DNA sequencing cloning, plasmid transformation and amplification[33]	Directed template enzymatic synthesis[35]	Attenuation, heat or chemicals[36]
Development Speed	Straight-forward production of peptides using automated synthesisers[37]	Can be immediately designed from microbial genomic sequences for synthesization[38]	Can be quickly synthesized for wide-scale production using genetic material[34]	Time-consuming process, lasting multiple years with an average timeline of roughly 11 years[39]
Safety	Cannot change to pathogenic form, composed of minimally immunogenic peptide sequences[31]	No toxic components, carries possible risk of genomic integration[35]	No toxic components, does not carry risk of genomic integration[35]	Weakened pathogens can pose risk to immuno-compromised individuals

Table 1: Comparative Features of Synthetic and Traditional Vaccines.

Synthetically produced peptide-based vaccines, which are one of the most prominent vaccine types, are being developed to target cancer cells. Developing vaccines contain multiple defined CD4+ and CD8+ T cell epitopes, which assists in overcoming genetic diversity, promoting direct activation of adaptive immunity, and inducing long-lasting immune response against cancer. Peptide-based vaccines carrying diverse epitopes carried in a large group of people can provide a wider range of protection, exposing the immune system to more strains and covering a broader portion of the population.[40] Importantly, it avoids original antigenic sin, which is when the immune system elicits a response to other viral infections only from the experience of the first viral variant, by displaying multiple peptide epitopes at once.[41] With its versatile approach, peptide-based vaccines can have the potential to prevent cancerous cells from escaping immune response. Through the vaccines, the presentation of the transported peptides is activated by dendritic cells on MHC class I and II molecules, which present to CD8+ and CD4+ T cells. After the T cells are activated, the CD8+ T cells go to tumor sites to kill tumors and the CD4+ cells assist the CD8+ cells, while fostering B cell activation.[42] Through clinical testing, patients with prostate cancer who received injections of a synthetic long vaccine targeting the Ros homolog gene family C, also known as RhoC, were able to generate ample CD4+ responses without detrimental effects. The synthetic vaccine presented 3 HLA-class II epitopes that were able to be recognized, and successful patients showed enduring immune responses, lasting at least 10 months.[43] Although there is much progress, HLA restrictions remain a large concern and must be addressed through finding effective epitopes to match HLA types and joining multiple peptides.

In addition, synthetic DNA vaccines have been investigated as a possible cancer immunotherapy for its ability to stimulate immune responses against cancer. In DNA vaccines, the plasmid DNA is injected into cells, which reaches the nucleus to start transcription into mRNA and translation into tumor antigen.[44] DNA vaccines have shown promise for their customizability. DNA vaccines are flexible in that genes can be edited to encode different types of antigens to induce immune response, and do not require toxic treatments. Through research, scientists determine the most appropriate genes to be used, and DNA sequences are located into a plasmid or viral vector to be transported into the patient.[44] In particular, DNA

vaccines designed with multi-antigen vaccination have shown favorable results in inducing T-cell responses. DNA vaccines can contain multiple sequences from different antigens, which can improve antigen immune response. A DNA vaccine developed with a DNA plasmid encoding multiple epitopes was received by patients, and the majority of patients were able to produce antigen-specific T-cell responses.[45] Another clinically developed DNA vaccine is STEMVAC, a plasmid-based DNA vaccine that can induce IFN-g-secreting T-cell responses in patients with triple-negative breast cancer.[46] DNA vaccines are also advantageous in their low cost and straightforward manufacturing process.[46] Concerns about the DNA vaccine arise from its ability to integrate into the genome, which can incur the risk of insertional mutagenesis.[46] Insertional mutagenesis increases the rate of mutations, which could affect the safety of the vaccine.[47] In addition, there is still much research needed to increase the immunogenicity of DNA vaccines through pairing of adjuvants, as most developed DNA vaccines are insufficient on their own.[47]

Synthetic mRNA vaccines have also emerged as a promising development in cancer immunotherapy. In mRNA vaccines, the vaccine introduces mRNA coding into somatic cells, which instructs cells to start translation and antibody production.[48] The speed of mRNA vaccines is an important therapeutic advantage, as the coding can be translated into proteins expeditiously without nuclear dependence. The mRNA vaccine skips transcription, starting translation.[48] By bypassing the nucleus, the mRNA vaccine expedites the process to rapidly produce peptides for stimulating immune response. It is also considered safer than DNA vaccines since it integrates into the cytoplasm instead of the nucleus, which prevents insertional mutagenesis from occurring.[48] After the protein is made, the vaccine RNA is degraded, which reduces the infection risk and increases the vaccine's stability.[49] Through testing, a mRNA vaccine concentrated with defined neoantigens, and epitopes was administered to patients with gastrointestinal cancer and produced positive results.[50] The vaccine was able to induce targeted T-cell responses against anticipated mutations and showed no adverse reactions in patients.[50] In another study, mRNA neoantigen vaccines engineered from pancreatic ductal adenocarcinoma tumors were treated to patients, and responsive patients produced vaccine-expanded T cells.[51] For mRNA vaccines to progress in becoming a primary cancer

immunotherapeutic treatment, there must be a reached agreement on its administration route. Currently, there is no common agreement on the ideal administration route for mRNA vaccines (intramuscular, intradermal, etc.). The administration route is important in affecting the mRNA vaccine's allocation and protein translation.[52]

4. Future Directions

Although many of these treatments represent a key advancement in medicine and pharmaceuticals, the implementation of these treatments to the general public is limited by a combination of economic, regulatory, and ethical challenges. Such barriers slow the integration of the synthetic therapies into mainstream healthcare and raise critical concerns about their long-term viability.

Accessibility is one of the largest obstacles that must be overcome. Interventions, such as CAR-T cell therapies, CRISPR-edited immune cells, and stem cell-based treatments, are prohibitively expensive for the majority of patients.[53,54,55] The high cost stems from various factors; there are complex, labor intensive manufacturing processes, strict quality control standards, and the cost of regulatory compliance associated with these treatments.[56] Essentially, it is difficult to produce these treatments at scale and even more costly to deliver them to patients. Additionally, insurance coverage for advanced therapies must be expanded in order to make care more affordable for patients in low-income settings or areas that lack robust healthcare infrastructure. There are disparities in access to novel treatment options in patients in high-resource environments compared to under-resourced regions.[57] Furthermore, regulatory frameworks pose another challenge to deliver treatment to the general public, especially for treatments that involve live cells or genome editing. CRISPR and other synthetic biology techniques raise concerns about long term safety and heritability. Somatic cell editing is designed to affect only the treated individual, but off-target effects and unintended consequences remain a concern.[58] Such interventions carry the risk of unintended multigenerational consequences and call for careful ethical scrutiny, particularly in the context of global regulatory asymmetries. In order to solidify the safety and efficacy of these treatments, clinical trials are to be done at a larger scale in order to strongly prove efficacy of

treatments. Many clinical trials examined in the paper are done at an initial phase with optimistic results but insufficient results for the treatments to be brought to the broad patient base. Clinical trials are expensive and capital intensive, which makes them a notable area of focus in terms of the next steps to be taken by researchers and institutes.

Despite the current challenges, there is growing optimism that recent innovations are beginning to address these barriers directly. Advances in manufacturing and automation are reducing production costs and improving scalability. Automated closed-system bioreactors operate in functionally closed environments and streamline production workflows while reducing contamination risk, though multi-patient scalability remains a key challenge.[59,60] Emerging strategies involving direct in vivo CAR-T cell engineering are redefining manufacturing needs. Lentiviral and lipid nanoparticle systems allow the patient's own T-cells to be genetically reprogrammed inside the body, bypassing labor-intensive ex-vivo manipulation and potentially eliminating the logistics and wait times that currently limit accessibility.[61,62] Allogeneic, off-the-shelf CAR-T therapies are advancing in clinical trials with 81% one-year survival in hematologic malignancies. Enabling multiple treatments per manufacturing bath and reducing per-patient cost.[63,64]

Synthetic gene circuits are enchanting specificity and tunability of engineered immune cells by requiring multiple tumor signals for activation.[65,66] Synthetic vaccines are showing durable T-cell responses and improved immunogenicity through personalized neoantigen approaches, with mRNA vaccines providing rapid production advantages.[67,68] Combination immunotherapy pairing CAR-T cells with checkpoint blockade has achieved superior survival outcomes compared to standard regimens. Engineering immune cells to sense hypoxia and metabolic signals in the tumor microenvironment is further improving infiltration and persistence.[70,71]

Artificial intelligence is accelerating patient selection and therapy optimization by predicting immunotherapy response more accurately than conventional biomarkers.[72,73] Prime editing and base editing technologies

now enable precise, scarless genetic modifications with reduced off-target effects.[74,75]

Innovations in synthetic biology are making therapeutic cells more programmable and resilient, and artificial intelligence is accelerating drug discovery and target identification.[76] The rise of precision medicine offers the potential to tailor treatments to individual genetic and immunological profiles, which may help minimize adverse effects and optimize outcomes.

Ultimately, addressing the economic, regulatory, and ethical challenges surrounding advanced cell-based therapies is essential to ensure their successful integration into mainstream healthcare. As scientific innovation accelerates, equal attention must be given to improving affordability, access, and oversight to realize the full potential of these treatments.

5. Conclusion

Engineered cell-based immunotherapies, synthetic gene circuits, and synthetic cancer vaccines have emerged as promising therapeutic approaches for a variety of cancer types. Synthetic biology methods can be utilized to improve the specificity, programmability, and efficacy of cancer immunotherapies, potentially overcoming the challenges of toxicity and long-lasting potency.

Engineered cell therapies demonstrate promising potential with regards to immunotherapies for solid tumors and various forms of cancer. There are several preclinical and clinical studies that have shown the efficacy of CAR-T cell therapies, CAR-NK cell therapies, HSC-INKT cell therapies, TCR-T cell therapies, and the use of pluripotent stem cells as immunotherapy. Synthetic construction of immune cells and stem cells as a way to combat the tumor microenvironment and metastasis is a field of synthetic biology that requires significant attention by researchers and the scientific community as it represents a creative and novel way to fight malignancies.

Recent studies demonstrate that synthetic gene circuits can greatly enhance cancer immunotherapy. Logic-gated receptor circuits and adaptable CAR platforms improve specificity. Autocrine cytokine circuits and inducible switches enable dynamic control of immune responses. Reprogramming of

macrophages and DCs broadens the cellular arsenal against tumors. Finally, CRISPR-based networks offer versatile control modules for precise tumor targeting. Together, these advances form a framework for designing smarter cell therapies tailored to the complex demands of solid tumors.

Synthetic vaccines, including peptide, DNA, and mRNA types, provide advantages over traditional vaccines in fast production, targeted editing, and safety. Peptide-based vaccines are developed using multiple T cell epitopes, which brings a broader scope of protection that can prevent cancer from evading the immune system. DNA vaccines can be modified to carry different genes that express a range of antigens, and mRNA vaccines can be quickly incorporated into cells without nuclear dependence. With further progress, these types of synthetic vaccines display potential for cancer treatment.

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