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REVIEW ARTICLE

Immune Modulation Strategies in Gene Therapy: Overcoming Immune Barriers and Enhancing Efficacy

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Abstract: The immune system presents significant obstacles to gene therapy, which has limited its use in treating many illnesses. New approaches are needed to overcome these problems and improve the effectiveness of gene therapy. This study explores several techniques to immune regulation within gene therapy, a cutting-edge discipline that aims to optimise results by fine-tuning the immune response. We cover new ways to control the immune system and deliver therapeutic genes just where they are needed, including influencing immunological checkpoints, causing immunotolerance, and making smart use of immunomodulatory drugs. In addition, the study provides insight into new developments in the design of less immunogenic gene delivery vectors, which allow for the extension of transgene expression with minimal adverse immune reactions. In order to maximise the efficacy of gene-based therapies, this review analyses these novel approaches and gives a thorough overview of the present state of the art by addressing obstacles and pointing the way toward future developments in immune regulation. Not only does their integration provide new opportunities for the creation of safer and more effective gene treatments, but it also contains the key to overcome current obstacles.

Keywords: Gene therapy, immune modulation, immunotolerance, immune checkpoints, immunomodulatory agents, gene delivery vectors.

1. INTRODUCTION

Gene therapy, which uses genetic modification to replace or fix defective genes, has great potential for treating a wide range of inherited and acquired illnesses. The complex and dynamic immune system, which may establish strong defences against therapeutic vectors, transgene products, and even modified cells, poses a serious obstacle to the practical translation of gene treatments [1]. The attainment of therapeutic effectiveness while avoiding immune identification is a significant obstacle to the effective use of gene treatments. This study explores the complex interactions between gene therapy and the immune system, with a particular emphasis on the tactics used to influence immune responses and improve the general effectiveness of gene-based therapies. By analysing the many immunological barriers that stand in the way of effective gene delivery and expression, along with the possible negative consequences of immune responses, our goal is to provide insight into novel strategies intended to overcome these obstacles [2]. The immune system's innate and adaptive components are essential for identifying and combating foreign substances, such as the viral vectors often used in gene therapy. Thus, understanding the processes through which the immune system identifies and eradicates these vectors is essential for formulating tactics that might maximise treatment results. Furthermore, it's critical to investigate all immune modulation strategies in order to prevent undesirable immune responses, since transgenic products have the potential to trigger immunological responses and have unintended consequences. In this thorough overview, we will examine current developments in immune modulation techniques, from creating viral vectors that elude immune detection to creating immunomodulatory medications that may reduce immunological responses [3].

We will also go over the importance of personalized medicine in adjusting gene therapy strategies to each patient's unique immunological profile in order to maximize therapeutic advantages and reduce the possibility of unfavourable

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outcomes. This study attempts to provide a critical synthesis of the status of immune modulation tactics at the moment, providing insights into the difficulties encountered by both researchers and physicians as the field of gene therapy quickly advances [4]. We can create safer, more efficient, and broadly applicable gene-based medicines for a wide range of disorders by comprehending the complex dance between gene therapies and the immune system. In the end, this information will transform the medical industry and enhance the lives of countless people. In the end, this information will transform the medical industry and enhance the lives of countless people by revolutionizing treatment options and improving patient outcomes [5]. Additionally, understanding the interplay between gene therapies and the immune system can help researchers address the potential side effects and adverse reactions that can arise from these treatments. By unravelling the intricate relationship between gene therapies and the immune response, researchers can develop strategies to minimize immune rejection and optimise the effectiveness of gene-based medicines [6]. This knowledge will not only pave the way for more successful gene therapy interventions but also open doors for innovative approaches in personalized medicine and targeted therapies. Ultimately, the advancements in this field have the potential to revolutionize healthcare and provide hope for individuals suffering from currently incurable diseases [7].

2. CHALLENGES IN ACHIEVING LONG-TERM GENE EXPRESSION: FOCUS ON IMMUNE CLEARANCE

In gene therapy, immune clearance is a major obstacle that must be overcome to achieve long-term gene expression. When it comes to gene therapy, the immune system's function is to identify and get rid of foreign materials, which include the transformed cells or vectors that are injected into the body [8]. The following are some major obstacles to immune clearance in the context of maintaining gene expression:

2.1. Immunogenicity of Viral Vectors

In the field of gene therapy, the immunogenicity of viral vectors is a crucial consideration. Viral vectors are often used to transfer therapeutic genes into target cells. The immunogenicity of viral gene transfer can stimulate an immune response against the therapeutic transgene product. This response may occur because of the specific type of gene mutation present, making patients with null mutations more likely to identify the transgene product as a foreign antigen. Although there are shared characteristics in the immune response to several viruses, each vector has its own array of activation signals, which are also influenced by the individual tissue's environment [9]. However, the immune system's response to these vectors may impact the efficacy and safety of gene therapy. Immunogenicity is the term used to describe the capacity of viral vectors used in gene therapy to provoke an immune response in the recipient. Viral vectors used for delivering therapeutic genes might potentially elicit both innate and adaptive immune responses. The adaptive immune system produces antibodies and cytotoxic T cells, whereas the innate immune system detects viral components, leading to inflammation. The capsid proteins of the vector are often targeted by the immune system for identification. The effectiveness of treatment may be hindered by pre-existing immunity resulting from prior exposure to the vector or closely similar viruses [10]. Methods to reduce the likelihood of an immune response include modifying the protein coat of the virus [11], using drugs that suppress the immune system [12], using different types of viruses [13], inducing immune tolerance [14], and investigating different ways of administering the treatment [15]. The goal is to achieve effective delivery of the therapeutic genes while minimising the immune system's clearance of the treatment, thus ensuring long-term gene expression in gene therapy applications [16].

2.2. Innate Immune Activation

One of the main components of the body's nonspecific, immediate defence systems against infections is innate immune activation. Immune cells' pattern recognition receptors (PRRs) identify conserved molecular patterns linked to foreign invaders like viruses and bacteria when the innate immune system identifies them. The initiation and regulation of innate immune responses are controlled by various types of genetically determined pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), Nod-like receptors (NLRs), AIM2-like receptors (ALRs), C-type lectin receptors (CLRs), and other DNA sensors [17]. When a pathogen invades, these pattern recognition receptors (PRRs) initiate the activation of NF-κB, type I interferon (IFN), or other inflammasome signalling pathways. This, in turn, results in the production of various proinflammatory and antiviral cytokines and chemokines, which subsequently induce adaptive immune responses. However, excessive innate immune activation can cause inflammation and the clearance of therapeutic drugs in the setting of gene therapy or the introduction of foreign materials, which presents difficulties for prolonged gene expression. The activation of the innate immune system facilitates the initiation of adaptive immunity by promoting the proliferation, differentiation, and survival of lymphocytes *via* the stimulation of cellular components of the innate immune system, such as dendritic cells and cytokines [18].

2.3. Adaptive Immune Responses to AAV Vectors

Adeno-associated virus (AAV) vectors are commonly used in gene therapy due to their ability to deliver genetic material to target cells with minimal immunogenicity. However, the host's adaptive immune system can still mount a response against AAV vectors, particularly through the recognition of AAV capsids and transgene-encoded proteins. When an AAV vector transduces a dendritic cell, the viral capsid proteins and transgene-encoded proteins can be processed and presented to the immune system. This process begins with the degradation of AAV capsids and transgene pro-

teins by the proteasome within the transduced dendritic cell. The proteasome degrades unneeded or damaged proteins into peptides, which are then transported into the endoplasmic reticulum and bind to major histocompatibility complex (MHC) molecules [19].

There are two classes of MHC molecules involved in antigen presentation: MHC class I and MHC class II. Peptides derived from the proteasome degradation of proteins are typically presented on MHC class I molecules, which are recognized by $CD8^+$ T cells (cytotoxic T lymphocytes). When a $CD8^+$ T cell receptor (TCR) recognizes a peptide-MHC class I complex, the $CD8^+$ T cell becomes activated, proliferates, and differentiates into effector cells that can kill infected or transduced cells displaying the same antigen [20].

Understanding these immune responses is crucial for improving the efficacy and safety of AAV-mediated gene therapies. Strategies to mitigate these responses include immunosuppressive regimens, capsid engineering to evade immune detection, and transient modulation of the immune system [21]. The overview of the adaptive immune responses AAV vectors is shown in Fig. (**1**). AAV capsids and transgene-encoded proteins within a transduced dendritic cell can be degraded by the proteosome and the resulting peptides are presented on MHCs leading to activation and proliferation of $CD4^+$ and $CD8^+$ T cells (Fig. **1**).

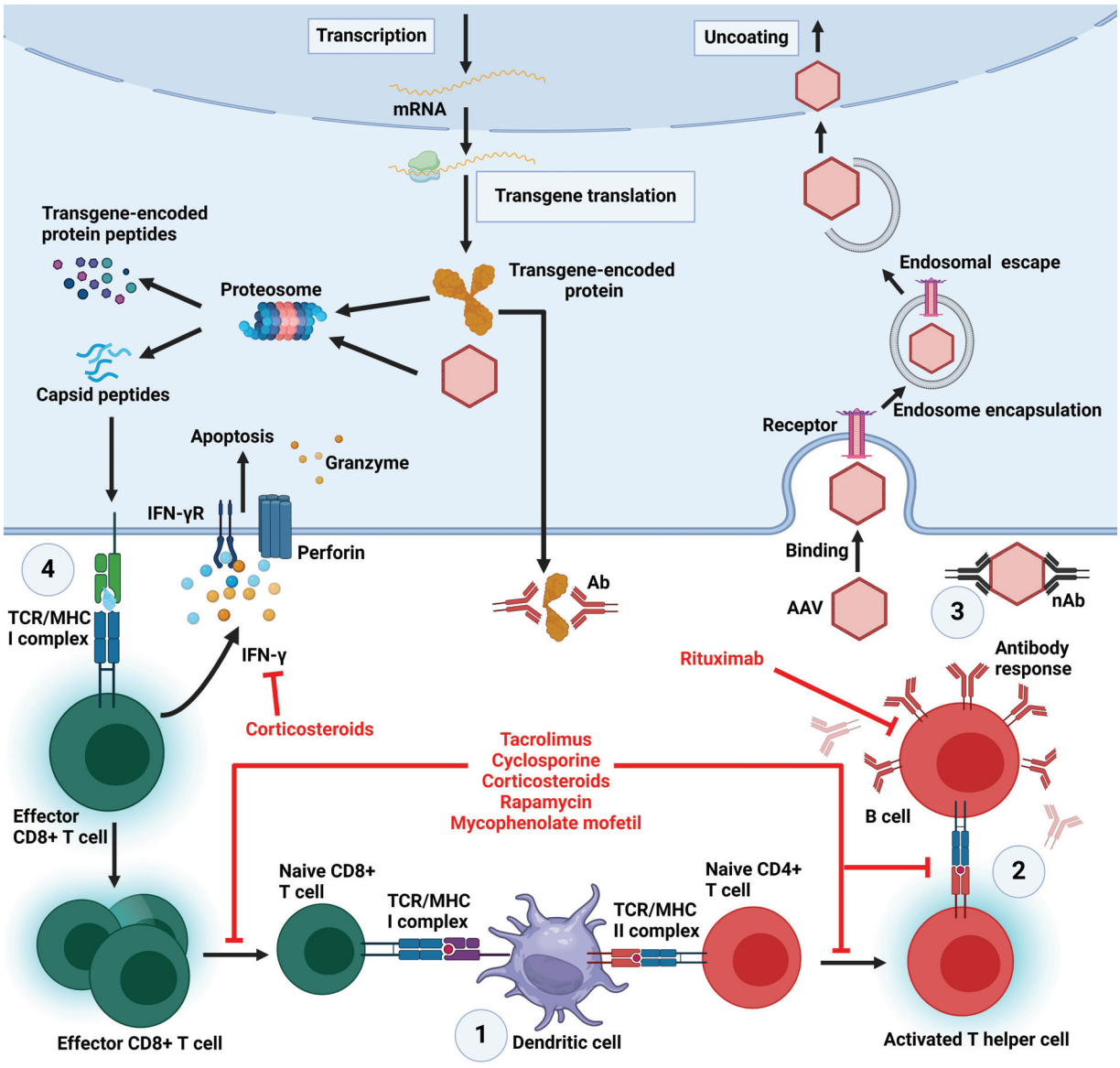

Fig. (1). Overview of the adaptive immune responses AAV vectors. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

2.4. Immune Memory and Repeat Administrations

Exploring immunological memory experimentally has shown to be challenging. While the ancient Greeks were the first to document this phenomenon, it has been regularly utilised in vaccination programmes for more than two centuries. However, it is only recently that we are beginning to understand that memory is maintained by a distinct population of specialised memory cells, which exist separately from the original antigen that triggered their formation. This memory maintenance mechanism aligns with the observation that only individuals who have been previously exposed to a specific infectious agent develop immunity, and that memory is not contingent on repeated exposure to infection through contact with other sick persons [22]. This phenomenon was deduced from studies conducted on isolated island populations, when a viral pathogen like measles might trigger an epidemic, affecting the whole resident population, only to thereafter vanish for an extended period. Upon reintroduction from external sources, the virus does not impact the original population but induces illness in individuals born after the initial outbreak. Therefore, it is not necessary to repeatedly expose oneself to the infectious virus in order to maintain immunological memory. Instead, it is most probable that memory is maintained by durable antigen-specific lymphocytes that were generated after the initial exposure and continue to exist until a subsequent encounter with the pathogen [23]. Previous beliefs suggested that the presence of antigen, which is trapped in immune complexes on follicular dendritic cells, played a vital role in the survival of these cells. However, current research indicate differently. Although the majority of memory cells remain in a quiescent state, meticulous research has revealed that a small fraction undergoes division at any given moment [24]. The factors that trigger this sporadic cell division remain uncertain. However, cytokines, whether produced constantly or throughout the progression of immune responses targeted at noncross-reactive antigens, may be accountable [25]. IL-15, a specific type of cytokine, has been found to play a role in preserving CD8 memory T cells. The amount of memory cells specific to a particular antigen is tightly controlled and remains rather stable during the memory phase, irrespective of cell division. A secondary immunological response will occur when the same antigen is encountered again. This has resemblance to the primordial immune response, wherein B cells and T cells undergo early growth at the interface between the T- and B-cell zones. The secondary reaction is distinguished by the prompt and robust development of plasma cells, which explains the early and abundant synthesis of IgG antibodies [26]. Undifferentiated B cells have the ability to move to the follicle and transform into germinal centre B cells. At this stage, the B cells experience a second phase of rapid growth, during which the DNA that encodes their immunoglobulin V domains undergoes somatic hypermutation. After this, the B cells develop into plasma cells that secrete antibodies. The antibodies generated by plasma cells during the initial and early subsequent immune responses play a crucial function in promoting the refinement of affinity during the subsequent immune response. During secondary and subsequent immune reactions, the B cells that developed in the first response produce persistent antibodies that can readily attach to the freshly introduced antigen [27]. Certain antibodies facilitate the redirection of antigens to phagocytes for the purpose of breaking them down and eliminating them. If there is an enough amount of preexisting antibody to eliminate or neutralise the pathogen, it is plausible that no immune response will occur. Nevertheless, in the presence of a small amount of antigen, B cells that have receptors capable of binding the antigen strongly enough to outcompete the existing antibody will capture the unbound antigen. They will then break it down into smaller peptide fragments and present these fragments, attached to MHC class II molecules, to activated helper T cells that are located in the germinal centres [28]. The interaction between B cells displaying antigenic peptides and armed helper T cells that are specific to the same peptide results in the exchange of activating signals and the fast multiplication of both activated antigen-specific B cells and helper T cells. Therefore, only the memory B cells with a stronger binding capacity are effectively activated during the secondary immune response. Thus, the antibody's affinity increases gradually because only B cells with high-affinity antigen receptors are capable of efficiently binding the antigen and being stimulated to multiply by antigen-specific helper T cells [29].

2.5. Limited Tissue Specificity, Risk of Insertional Mutagenesis and Pre-existing Immunity

One barrier in gene therapy is limited tissue specificity, which refers to the difficulty of accurately directing and restricting therapeutic gene expression to particular tissues. Gene therapy should ideally target the damaged tissues specifically, with the least amount of effect on healthy tissues. However, achieving this specificity can be challenging. Non-targeted expression in non-diseased tissues may trigger unintended immune responses, leading to the clearance of modified cells and potentially compromising the safety and efficacy of the therapy [30]. Researchers are investigating the use of tissue-specific promoters, which predominantly stimulate gene expression in the appropriate tissues, as a solution to this problem. Ensuring that the therapeutic effects are concentrated in the desired target tissues, efforts are being made to improve the safety and precision of gene therapy by fine-tuning the control over where therapeutic genes are produced [31].

When integrating vectors are employed in gene therapy, there is a risk of insertional mutagenesis. The process of integrating the therapeutic gene into the host genome, known as insertional mutagenesis, may cause unexpected genetic changes and interfere with normal gene function. Even while this integration is necessary for ongoing gene expression, the therapeutic gene's random insertion could damage important genes or regulatory components, which could have negative consequences including unchecked cell division or disruption of biological functions [32]. To reduce this risk, scientists are creating safer vector systems that allow for site-specific integration and reduce the possibility of harmful genetic changes. The objective is to guarantee the safety and long-term stability of gene therapy treatments by

mitigating the danger of insertional mutagenesis, hence decreasing the probability of inadvertent outcomes linked to genomic integration [33].

One important aspect affecting the effectiveness of gene therapy is pre-existing immunity, which is the existence of immune responses in people as a result of previous exposure to particular viral vectors or related viruses. Natural infections, immunizations, or contact with the same or closely related viral vectors used in gene therapy can all contribute to this pre-existing immunity [34]. Gene therapy outcomes can be greatly impacted by the presence of preexisting antibodies or memory T cells that are capable of recognizing these vectors in a person. After being administered, these immunological components can quickly destroy the viral vectors, preventing them from delivering therapeutic genes to the intended cells. This recognition can also lead to a heightened immune response, potentially causing inflammation and clearance of the modified cells (Table **1**) [35].

3. STRATEGIES FOR IMMUNE EVASION IN GENE THERAPY

Immune evasion techniques are essential in gene therapy to get over the obstacles presented by the immune system in identifying and eliminating therapeutic vectors and altered cells [36]. Various strategies have been investigated to improve the efficacy of gene therapy through immune response evasion:

3.1. Capsid Engineering

Capsid engineering is used to specifically direct the cell surface with the aim of altering or broadening the tropism. Additionally, it is used to enhance the internal cellular processing and to detect and modify immunogenic epitopes. Engineering focuses on well-founded assumptions or methodical design when there is available knowledge on how to address the challenge. In all other cases, it is more advantageous to apply changes to the capsid in a random way, and then employ high throughput selection screens on capsid libraries. Both methods need the use of appropriate assay equipment, and it is recommended to give priority to *in vivo* models [37]. In this specific situation, the mice models that have been modified to resemble humans seem to be the most effective choice for improving AAV vectors for liver diseases in people. Nevertheless, it is crucial to acknowledge that these models do not provide a comprehensive comprehension of the host's response to AAV. Hence, more work is required to develop enhanced animal models. An essential strategy in the domain of gene therapy is capsid engineering, which alters the outer shell or capsid of viral vectors to enhance their effectiveness and evade immune detection. The capsid is both a significant target for the host immune system and a crucial vehicle for delivering therapeutic genes to target cells. Scientists can reduce the immunogenicity of the capsid, meaning they can make it less recognisable to the immune system, by modifying certain regions of the worm [38].

This process involves identifying and modifying certain regions on the outer surface of the capsid that are often recognised by neutralising antibodies. Antibodies bind to certain regions called epitopes. Through the use of genetic engineering, researchers may meticulously alter these epitopes to create capsids that have a reduced likelihood of being detected by antibodies. This will enhance the viral vector's ability to evade immune detection [39]. Capsid engineering is crucial in order to surmount immunological obstacles that would otherwise hinder the efficacy of gene therapy. Furthermore, this strategy not only reduces the likelihood of neutralising antibody responses, but also enhances the stability of the vector and augments its potential to deliver therapeutic genes to specific cells. Ongoing advancements in capsid engineering permit the development of more potent and immunologically stealthy viral vectors, ultimately enhancing the safety and efficacy of gene therapy treatments [40].

Table 1. Summarizing challenges in achieving long-term gene expression with a focus on immune clearance.

Challenge	Description
Immunogenicity of Viral Vectors	Viral vectors used for gene delivery can trigger immune responses, leading to the clearance of transduced cells and limiting gene expression.
Host Immune Response	The host's immune system can recognize and eliminate transgene-expressing cells, hindering sustained gene expression.
Innate Immune Activation	Activation of innate immune responses, such as interferon pathways, can lead to rapid clearance of transduced cells.
Adaptive Immune Memory	Memory T cells may form against viral vectors or transgene products, resulting in a faster and more robust immune response upon re-exposure.
Antibody Neutralization	Pre-existing or developed antibodies against viral vectors can neutralize them, reducing the effectiveness and longevity of gene expression.
Inflammatory Reactions	Inflammation at the injection site or in transduced tissues can contribute to immune cell infiltration and clearance of gene-modified cells.
Tissue-Specific Immune Responses	Immune responses may vary in different tissues, with some being more prone to inflammation and immune-mediated clearance.
Integration Site Effects	Random integration of viral vectors into the host genome may lead to unintended consequences, including activation of nearby immune genes.
Dosing and Vector Selection	Optimal dosing and choosing appropriate vectors are critical for minimizing immune responses and achieving sustained gene expression.
Gene Silencing Mechanisms	Cellular mechanisms like RNA interference may downregulate transgene expression over time, reducing long-term efficacy.
Re-administration Challenges	Re-administering gene therapy may be challenging due to the development of anti-vector immunity, limiting the potential for repeat treatments.

3.2. Immunosuppression

In the context of gene therapy, immunosuppression is a therapeutic tactic used to temporarily reduce or modify the immune response. This strategy seeks to lessen the immune system's ability to identify and eliminate therapeutic vectors or altered cells, so fostering an environment that will support the success of gene therapy. Gene therapy may be used in conjunction with immunosuppressive medications, such as corticosteroids or other immunomodulatory medicines, to reduce the activity of immune cells [41]. Immunosuppression aims to extend the duration of therapeutic vectors and the expression of transgenes within target cells by inhibiting the immune response. This is especially important when working with viral vectors since, after repeated exposure, the immune system can establish powerful and quick defences against them. The use of immunosuppression, however, must be carefully considered as it may impair immunological function overall, making a person more vulnerable to infections or other immune-related problems [42]. Immunosuppression, despite its possible advantages, is frequently a temporary solution meant to open a window of time for gene therapy's therapeutic effects to manifest before the immune response resurfaces. Optimizing the safety and efficacy of gene therapy interventions requires striking the correct balance between attaining efficient immune evasion and preserving the body's defences against infections. Research is still being done to improve immunosuppressive tactics so that side effects are reduced and long-term gene therapy outcomes are better accommodated [43].

3.3. Immune Tolerance

To mitigate the immunological reaction against therapeutic molecules, one strategic approach in gene therapy is the establishment of immune tolerance. In the context of gene therapy, promoting immunological tolerance aims to desensitize the immune system to inserted vectors or transgenic products, which the immune system may perceive as foreign. This entails using a variety of strategies to encourage immune cells to become indifferent to or accepting of the therapeutic components. The primary role of the immune system is to identify and eradicate foreign antigens and cancerous cells, while simultaneously preserving immunological tolerance towards its own constituents [44]. Nevertheless, uncontrolled activation of the immune system can result in various clinical problems such as autoimmune diseases, solid organ transplantation (SOT), hematopoietic stem cell transplantation (HSCT), and allergy diseases. By eliciting immunological tolerance, specifically establishing tolerances to pathogenic immune cells, the body can prevent inflammation while maintaining its typical immune response to foreign substances. Immunological tolerance is established through the regulation of self-reactive T cells in both the thymus and the periphery. This regulation is referred to as central immunological tolerance and peripheral immune tolerance, respectively. During the process of positive selection in T cell development in the thymus, T cells that can recognise their own major histocompatibility complex (MHC) molecules are retained, while T cells with a high affinity for self-peptides are subsequently eliminated through negative selection [45]. Nevertheless, certain self-reactive T cells have the ability to evade the process of negative selection, hence posing a possible threat of autoimmune response. Peripheral immunological tolerance is necessary to restrict the reaction of these self-reactive T cells and prevent inappropriate activation of the immune system. Chronic exposure to antigens is necessary to control self-reactive T cells by rendering them functionally inactive (T cell incompetence and T cell deficiency) and promoting the development of regulatory T cells (Tregs). This process is crucial for establishing peripheral T cell tolerance. Dendritic cells (DCs) play a crucial role in the process of immunological tolerance by integrating different immune signals within the body [46]. They achieve immunological homeostasis restoration by triggering programmed cell death in inflammatory T cells, regulating both pro- and anti-inflammatory reactions, and promoting the expansion of immunomodulatory Tregs. In order to effectively treat diseases caused by excessive activation, it is crucial to promptly implement measures that preserve the dynamic equilibrium and functionality of the immune system. Immunosuppressive medicines are commonly employed to treat autoimmune illnesses and transplantation. However, it is important to note that these drugs necessitate long-term usage and do not provide a cure for the disease. Furthermore, prolonged use of immunosuppressive medications can lead to neurological, haematological, renal, gastrointestinal, and immunological damage [47]. Additionally, these side effects have the potential to compromise the body's innate immune response and heighten susceptibility to cancer and infection. Consequently, scientists are directing their attention towards treatments that promote immunological tolerance by specifically targeting immune cells. Immunotherapies can induce antigen-specific immunological tolerance without affecting other immune functions, leading to symptom relief and even disease cure [48]. Regulatory T cells (Tregs) are a useful tool for inducing immunological tolerance since they are essential in dampening hyperbolic immune responses. Regulatory T-cell administration or substances that increase their activity are examples of strategies that can be used to create an environment where the immune system is less prone to initiate aggressive responses against expressed transgenes or therapeutic vectors [49].

In order to allow for prolonged gene expression and persistent therapeutic benefits, researchers hope to lower the risk of immune-mediated clearance of therapeutic drugs by developing immunological tolerance. This strategy is especially crucial when administering gene therapy repeatedly because of the possibility of immunological memory and faster clearance. To maximize the safety and effectiveness of gene therapy interventions, induction of immunological tolerance presents a viable path—current research endeavours to improve and create novel approaches for accomplishing efficient and focused immune regulation [50].

3.4. Local Delivery

In contrast to systemic distribution, which distributes therapeutic medicines throughout the entire organism, local

delivery in gene therapy refers to the administration of therapeutic chemicals locally to a specific target tissue or region within the body. This strategy is used to reduce exposure to the immune system in circulation and to improve the accuracy of gene therapy in focusing on particular organs or tissues [51]. Local gene therapy administration concentrates therapeutic vectors and transgenic products in the targeted region, decreasing the possibility of systemic immune detection and clearance. Targeting specific tissues or organs impacted by a genetic abnormality or disease is especially beneficial with this approach since it enables more targeted and effective administration of therapeutic medicines [52]. Using devices made to release therapeutic vectors at the desired location, introducing gene-modified cells into the target tissue, or injecting directly into the tissue are examples of local delivery techniques. With systemic delivery, there is less chance of off-target immune reactions, and this strategy not only enhances therapeutic benefits in the target area. The specificity of the disease and the target tissue's accessibility determine whether local delivery is feasible, even though it offers benefits in terms of precision and decreased immunological exposure. In an effort to maximize the security and effectiveness of gene therapy therapies, researchers are still investigating and improving local delivery strategies [53].

3.5. Non-viral Vectors

Non-viral vectors are a different way to gene therapy that uses delivery mechanisms without using any viral components. Non-viral vectors include a wide variety of delivery techniques that are typically regarded as safer as and less immunogenic than viral vectors, which depend on altered viruses to transfer therapeutic genes into target cells. Various techniques, such as physical or chemical procedures using synthetic or natural chemicals, have been developed to aid in gene transfer [54]. The identified limitations of viral vectors, such as the occurrence of insertional mutagenesis and their tendency to provoke an immune response, have prompted a renewed focus on the advancement of non-viral vectors. Non-viral techniques have been used in over 17% of gene therapy studies within the previous decade. Lipid-based nanoparticles, polymer-based carriers, and other artificial or natural materials are examples of non-viral vectors. These vectors are designed to carry therapeutic DNA or RNA into target cells more easily by encasing and shielding it. Non-viral vectors lower the likelihood of immunogenic reactions and pre-existing immunity, which are frequently linked to viral vectors, by not including any viral components [55].

Non-viral vectors also have the advantage of being easily produced, having a variable payload size, and having the ability to be administered again. Ongoing research aims to enhance the effectiveness and design of non-viral delivery methods, even though they might not reach the high transduction efficiency of some viral vectors. The objective of improving safety and lowering the danger of immunological reactions is in line with the use of non-viral vectors in gene therapy. A viable path forward for gene therapy is the active exploration of new materials and formulations by researchers to maximize the stability, therapeutic effect, and efficiency of the distribution of non-viral vectors [56].

4. ENHANCING GENE THERAPY EFFICACY THROUGH IMMUNE MODULATION

The immune system consists of an intricate interconnected network of many cell types that work together to defend the body's tissues against additional infection and activate a group of specialised agents designed to precisely eradicate the invading pathogen. The cellular network can be broadly divided into two components: the innate arm and the adaptive arm. The innate immune responses begin at an early stage, are not specific to antigens, and do not lead to the development of immunological memory. The adaptive immune responses are influenced by the inflammatory milieu produced by the innate immune sensing [57]. These responses depend on the activation and clonal growth of antigen-specific B and T cells, which differentiate into effector cells. Additionally, they also build immunological memory. Viral vectors possess certain similarities to natural viruses, but they differ significantly in that they are non-replicative, administered in a concentrated dose, and introduced at an atypical location. While traditional immunological ideas can be utilised, it is important to note that the immune response to viral vectors also possesses distinct and inherent characteristics. Antibodies can neutralise vector particles carrying viral proteins that are the same or similar to antigens encountered by people during natural infection [58]. This can occur when these particles are injected into individuals who already have immunity. The detection of viral components such as capsids or nucleic acids by the innate immune system can lead to the infiltration of immune cells into tissues. This detection also stimulates the production of interferon (IFN)-α/β, also known as type 1 IFN or T1 IFN, which activates an antiviral state in the tissue, reducing the spread of the virus. Additionally, it serves as a signal to activate the adaptive immune response. The activation and subsequent presentation of antigens by dendritic cells (DCs) is a crucial process that connects innate and adaptive immunity [59]. This process results in the activation, differentiation, and proliferation of T lymphocytes. MHC class I-restricted $CD8^+$ T cells, also known as cytotoxic T lymphocytes (CTLs), have the ability to destroy cells that are infected with a virus. On the other hand, MHC class II-restricted $CD4^+$ T cells play a role in enhancing the activation of $CD8^+$ T cells and B cells, which leads to the production of antibodies. T helper (Th) cells play a crucial role in the development of memory responses. The Ad virus was among the initial viruses studied as a possible gene therapy vector. It was also the focus of early unsuccessful attempts at *in vivo* gene transfer, which emphasised the crucial role of host inflammatory responses in determining the long-term effectiveness of therapeutic gene expression and the overall safety of this intervention [60]. The initial excitement surrounding Ad vectors stemmed mostly from their exceptional transduction efficiency and packing capability. Nevertheless, the strong expression of the transgene was accompanied by a correspondingly intense inflammatory reaction, leading to temporary expression and a risk of severe immunotoxicity, ultimately culminating in the patient's demise. Due of their proficient capacity to activate $CD8^+$ T cells, future endeavours focused on employing them

as vaccine carriers and in cancer gene therapy. Ad vectors consist of a double-stranded DNA genome, approximately 36 kilobases in size, enclosed within a protein capsid of a viral origin. The virus replication is rendered faulty by removing many viral genes. Additionally, it is feasible to eliminate viral coding regions and generate "gutted" or "helper-dependent" adenoviral vectors. AdHu5 serotype effectively transduces several cell types *in vivo*, especially hepatocytes, but other serotypes that infect hematopoietic cells have also been documented [61]. After transduction, the vector genome persists as an episome. Ad vectors efficiently stimulate a wide range of innate immune pathways, making them valuable tools for investigating the innate immune response to viruses. Hepatic gene transfer is accomplished through the intravenous administration of an adenoviral vector. Nevertheless, immediate and instinctive reactions might manifest within a short timeframe of minutes to hours, resulting in alterations in blood pressure, a decrease in platelet count, inflammation, and an increase in body temperature. Three Coagulation dysregulation can extend to other organs and result in DIC (disseminated intravascular coagulation). Ad vectors stimulate vascular endothelial cells, leading to the secretion of von Willebrand factor (vWF) in the form of ultra-large-molecular-weight multimers [62]. vWF is an essential blood protein that plays a crucial role in platelet adhesion. Ad vectors additionally stimulate platelets and trigger the presentation of the adhesion molecule P-selectin, leading to the creation of platelet-leukocyte aggregates. This ultimately results in thrombocytopenia, increasing the risk of bleeding. Four early systemic administration of the Ad vector leads to crucial cellular interactions involving vascular and hepatic endothelial cells, platelets, Kupffer cells, hepatocytes, and splenic macrophages (MFs) and DCs. Shayakhmetov and colleagues have demonstrated that after the virus enters the bloodstream, the hexon component of the adenoviral capsid attaches to coagulation factor X (FX). Viral particles coated with FX stimulate Toll-like receptor (TLR)4 on the surface of splenic macrophages, leading to the activation of nuclear factor κB (NF-κB) and subsequent release of interleukin (IL)-1β [63]. This attracts polymorphonuclear leukocytes to the marginal zone of the spleen. The immune and coagulation systems have evolved together to protect against viruses, and these mechanisms help to quickly remove the virus from the spleen. Upon introduction into a blood vessel, the response to Ad vectors is significantly influenced by interactions with chemicals and cells present in the blood and immunological organs that monitor the systemic circulation. In addition to binding to coagulation proteins containing γ-carboxyglutamic acid (GLA) domains, adenoviral particles also attach to complement component C3 and natural immunoglobulin (Ig)M antibodies. This interaction leads to the activation of neutrophils, among other effects [64]. Macrophages can be stimulated to produce inflammatory cytokines and chemokines in response to antibody-virus complexes by activating the intracellular antibody receptor TRIM21. Remarkably, the attachment of FX seems to clash with adenoviral connections to complement and antibodies, safeguarding it from these elements while simultaneously encouraging TLR4 signalling in the spleen.Seventeen Ad vectors also engage with shed cellular receptors, necessitating further comprehensive investigations to determine their impact on immune responses. These tactics add to a complete immune modulation toolset intended for use in gene therapy [65]. Through the careful balancing of immune response activation and repression, researchers aim to maximize gene therapy interventions' safety, durability, and effectiveness for a variety of uses. The continued development of immune modulation methods has enormous potential to increase gene therapy's effectiveness and broaden its therapeutic uses [66].

4.1. Immunosuppressive Drugs

In the early stages, AAV gene therapy trials employed a responsive method of administering corticosteroids to address occurrences of increased liver enzymes that indicated liver damage. These instances were often thought to be linked to a specific immune response involving AAV capsid-specific cytotoxic T cells. Administration of corticosteroids usually resolves the increase in liver transaminase levels. Based on these discoveries, future clinical trials included preventive immunosuppression protocols that involved the administration of one or more pharmacotherapies [67]. Corticosteroids such as prednisone, prednisolone, and methylprednisolone attach to glucocorticoid receptors and alter the process of transmitting genetic information, leading to widespread anti-inflammatory and immunosuppressive effects. Corticosteroids achieve these effects by employing many mechanisms, such as reducing the expression of TLR (Toll-like receptor), inhibiting proinflammatory cytokines, and increasing the production of anti-inflammatory cytokines. Additional immunosuppressants employed in AAV gene treatments comprise rapamycin (sometimes referred to as sirolimus), MMF, calcineurin inhibitors (cyclosporine, tacrolimus), and rituximab. Rapamycin suppresses the proliferation of cytotoxic T cells and the differentiation of T helper cells by inhibiting the cell-cycle kinase called mammalian target of rapamycin [68]. At higher doses, it also suppresses the proliferation and differentiation of B cells. Azathioprine and MMF are antimetabolites that hinder the activity of inosine monophosphate dehydrogenase, which is the enzyme responsible for the production of guanosine nucleotides. This enzyme is more active in activated lymphocytes, so by inhibiting it, the proliferation of T and B cells is suppressed. Cyclosporine and tacrolimus hinder the activity of the signalling phosphatase calcineurin, which ultimately results in the inhibition of IL-2 transcription. This transcription is crucial for the proliferation of T cells, the maturation of regulatory T cells, and the expansion and cytotoxic effects of effector T cells. Rituximab, a monoclonal antibody, restricts the generation of antibodies by specifically targeting CD20 on B cells, hence triggering death. Hydroxychloroquine is being investigated as a potential treatment in early-stage trials [69]. It works by blocking the binding of TLR9 ligands and the subsequent signalling, which helps to limit the activation of T cells and the release of proinflammatory cytokines mediated by TLR. It is crucial to evaluate the safety characteristics of immunosuppressants to ensure that the

mitigation method does not lead to any extra negative effects. The overall safety profile of each immunosuppressant is influenced by factors such as dosage, treatment schedule, and duration of treatment. These factors also contribute to the occurrence of the most frequently observed adverse events associated with each immunosuppressant. Furthermore, individuals with weakened immune systems are particularly vulnerable to bacterial, fungal, and viral infections. Therefore, it is crucial to closely monitor and use a proactive approach to prevent or effectively manage infectious occurrences during immunosuppressive treatment [70].

4.1.1. Corticosteroids

Corticosteroids, by interacting with the glucocorticoid receptor, have a vital function in regulating the immune response by decreasing the synthesis of proinflammatory cytokines and chemokines. The anti-inflammatory mechanism of corticosteroids has rendered them effective in the treatment of diverse ailments, such as autoimmune disorders and inflammatory diseases. Nevertheless, the prolonged utilisation of these substances is linked to substantial detrimental consequences. Extended administration of corticosteroid medication has been associated with problems such as osteoporosis, metabolic disruptions, and an elevated susceptibility to cardiovascular disease [71]. Osteoporosis is caused by the detrimental effects of corticosteroids on bone density, resulting in a heightened vulnerability to fractures. Metabolic disruptions, such as changes in glucose metabolism, can play a role in the development of illnesses like diabetes. Moreover, the increased susceptibility to cardiovascular illness is ascribed to the drug's capacity to cause high blood pressure, abnormal cholesterol levels, and encourage a tendency for blood clot formation. It is important to carefully weigh the positive advantages of corticosteroid treatment against the potential negative effects. This requires close monitoring and consideration of other treatment alternatives, if available [72].

4.1.2. Rapamycin, or Sirolimus

Rapamycin, or sirolimus, predominantly suppresses the immune system by inhibiting the mammalian target of rapamycin (mTOR). This inhibition leads to the suppression of the activation of cytotoxic T cells and helper T cells, the regulation of the formation of regulatory T cells (Tregs), and the decrease in the proliferation and differentiation of B cells and T cells. Although rapamycin has demonstrated its efficacy in avoiding organ rejection in transplant recipients and managing specific autoimmune disorders, it is not devoid of adverse reactions. Rapamycin use is linked to adverse responses, one of which is thrombocytopenia. Thrombocytopenia refers to a decrease in platelet numbers, which might result in an increased tendency to haemorrhage [73]. Dyslipidemia, which refers to abnormal lipid levels, is a significant worry that could potentially contribute to complications related to the cardiovascular system. Mucositis, hindered wound healing, and proteinuria (abnormal levels of protein in the urine) are supplementary adverse effects that can present difficulties during the course of treatment. However, it is essential to effectively control the dosage and manage the side effects of rapamycin in order to utilise its therapeutic advantages while mitigating the related hazards in clinical environments [74].

4.1.3. Hydroxychloroquine

Hydroxychloroquine inhibits TLR9-mediated responses to viral DNA *via* modulating Toll-like receptor 9 (TLR9). Furthermore, it disrupts lysosomal function, thus inhibiting the process of major histocompatibility complex (MHC)-mediated antigen presentation. Hydroxychloroquine has the potential to be used as a therapeutic agent in several circumstances, such as specific autoimmune illnesses, and is being investigated as a treatment for viral infections. Nevertheless, the utilisation of hydroxychloroquine is linked to a range of negative consequences. Patients frequently report gastrointestinal symptoms, such as nausea and diarrhoea, which can affect their ability to tolerate the treatment [75]. Extended usage of hydroxychloroquine is linked to more severe complications such as retinopathy, which affects the eyes, and cardiomyopathy, a condition that affects the heart muscle. Furthermore, there may be noticeable impacts on the conduction of electrical signals in the heart, highlighting the need for vigilant observation and a thorough evaluation of the potential risks and benefits before including hydroxychloroquine into a therapy plan. It is crucial to consider both the positive impacts and negative consequences in order to maximise the effectiveness of treatment and ensure the safety of patients [76].

4.1.4. Immunostimulatory Agents

Strategies for gene therapy that aim to improve the immune response to therapeutic interventions must include immunostimulatory drugs. Immunostimulatory treatments are intended to increase and maximize the activity of the immune system, in contrast to immunosuppressive medications, which reduce immunological responses. This is especially true when it comes to cancer gene therapy or vaccinations. Adding adjuvants—substances added to gene therapy formulations that boost the immune response—is one such strategy. In order to generate a stronger adaptive immune response against the therapeutic target, adjuvants activate the innate immune system [77]. These substances aid in enhancing the immune system's ability to identify viral vectors or transgenic products, which may result in more effective and long-lasting therapeutic effects. Gene therapies also makes use of another type of immunostimulatory drugs called cytokines, which include interleukins and interferons. These signalling molecules can be employed to alter the activity of the immune system and serve important functions in controlling immunological responses. For example, in cancer gene therapy, the immune system's capacity to identify and eradicate cancer cells may be boosted by the administration of certain cytokines. Using immunostimulatory drugs aims to increase the therapeutic effect of gene therapy by utilizing the body's innate defences [78]. Researchers want to increase the efficacy of treatments by deliberately inducing a stronger immune response. This is especially useful in situa-

tions where an engaged immune system is advantageous, like when cancer cells are targeted for elimination. Overly aggressive immune stimulation, however, might have unfavourable effects, so the precise formulation and administration of immunostimulatory drugs must be carefully considered. In the rapidly changing field of gene therapy, researchers are constantly investigating and improving the usage of these medicines to achieve a balance between enhancing the immune response and guaranteeing patient safety (Table **2**) [79-94].

5. ADVANCEMENTS IN IMMUNE MODULATION TECHNIQUES

5.1. Precision Immunomodulation

One innovative strategy in the field of gene therapy is precision immunomodulation, which aims to precisely target and adjust immune responses. This approach acknowledges the intricacy of the immune system and aims to modify particular elements or channels without causing a widespread and non-specific inhibition of immune performance. For gene therapy interventions to be as safe and effective as possible while reducing the possibility of unexpected side effects, precision immunomodulation is essential [95]. Precision immunomodulation has been made possible by developments in our knowledge of the intricate molecular and cellular mechanisms underlying immune responses. Methods for modulating specific immune cell populations or signalling pathways involved in immune activation and regulation are being investigated by researchers. With this strategy, the immune system can be more precisely influenced, maintaining its capacity to fight off infections while moderating gene therapy-related reactions only in certain areas [96].

The development of treatments that may specifically activate or suppress particular immune cell types, such as regulatory T cells (Tregs) to increase tolerance or cytotoxic T cells to boost antitumor responses, is a crucial component of precision immunomodulation. Furthermore, the use of synthetic molecules, like bogus receptors or antibodies, permits interference with particular immunological checkpoints or signalling molecules, offering accurate regulation of immune responses [1]. This development is especially important for tackling the problems with immune reactions to viral vectors and transgenic products used in gene therapy. Through precise immune modulation, scientists hope to find a fine balance that would maximize beneficial impacts on the immune system as a whole while guaranteeing successful treatment outcomes. It is expected that further research on precision immunomodulation will have a major impact on the development of safer and more customized gene therapy interventions in the future [97].

Table 2. List of drugs for immune modulation with mechanism of action and examples.

S. No	Drug	Mechanism of Action	Examples	Doses	Experimental Models	References
1	Cyclosporine	Calcineurin inhibitor	Sandimmune, Neoral	2-5 mg/kg/day (oral)	Rodents, primates	[80]
2	Tacrolimus	Calcineurin inhibitor	Prograf, Advagraf	0.1-0.2 mg/kg/day (oral)	Rodents, primates	[81]
3	Sirolimus	mTOR inhibitor	Rapamune	2-5 mg/day (oral)	Rodents, non-human primates	[82]
4	Mycophenolate mofetil	Inosine monophosphate dehydrogenase inhibitor	CellCept	1-1.5 g twice daily (oral)	Rodents, primates	[83]
5	Azathioprine	Purine synthesis inhibitor	Imuran	1-3 mg/kg/day (oral)	Rodents, non-human primates	[84]
6	Prednisone	Glucocorticoid	Deltasone, Rayos	5-60 mg/day (oral)	Rodents, primates	[85]
7	Belatacept	Costimulation blocker	Nulojix	5 mg/kg IV on day 1, 10 mg/kg on day 5 and 14, then 5 mg/kg every 2 weeks (IV)	Non-human primates, humans	[86]
8	Basiliximab	IL-2 receptor antagonist	Simulect	20 mg on day 0 and 4 (IV)	Humans	[87]
9	Daclizumab	IL-2 receptor antagonist	Zenapax	1 mg/kg on day 0, 14, 28 (IV)	Humans	[88]
10	Rituximab	CD20 antibody	Rituxan	375 mg/m^2 weekly for 4 doses (IV)	Humans	[89]
11	Alemtuzumab	CD52 antibody	Lemtrada	12 mg/day for 5 days (IV)	Humans	[90]
12	Thymoglobulin	Polyclonal antithymocyte globulin	Thymoglobulin	1.5 mg/kg/day for 5-7 days (IV)	Rodents, primates, humans	[91]
13	Atg-Fresenius	Lymphocyte depleting agent	ATG-Fresenius	9-16 mg/kg total dose (IV)	Rodents, primates, humans	[92]
14	Tofacitinib	JAK inhibitor	Xeljanz	5 mg twice daily (oral)	Rodents, non-human primates, humans	[93]
15	Fingolimod	Sphingosine-1-phosphate receptor modulator	Gilenya	0.5 mg once daily (oral)	Rodents, non-human primates, humans	[94]

5.2. Cell-Based Therapies

The goal of developments in cell-based therapeutics is to control immune responses and promote tolerance. This includes the use of immune cells such as regulatory T cells (Tregs) and others. For example, engineered Tregs can be made to suppress undesirable immunological responses, providing a more focused and regulated method of immunomodulation [98].

5.2.1. T Cells

T cells originate from hematopoietic stem cells in the bone marrow and are transported to the immunological organs and tissues of the body *via* lymph and blood circulation in order to carry out their immune tasks. T cells may be categorized based on their primary roles as cytotoxic T cells, helper T cells, regulatory/suppressor T cells, and memory T cells. CTLA-4/PD1-based immune checkpoint blockade (ICB) treatment and chimeric antigen receptor T-cell (CAR-T) therapy have been created and have gained remarkable success in medical practice by stimulating T cell function [99]. The process of CAR-T cell therapy primarily entails the retrieval of T cells from patients and their subsequent modification using genetic engineering techniques to express receptors capable of identifying tumour cells. The modified T cells are cultured in a laboratory setting and then reintroduced into the patient's body. Recently, CAR-T cell therapy has shown remarkable efficacy in the treatment of hematologic malignancies and solid neoplasms. An example of these is CAR-T cells that specifically target the CD19 receptor on B lymphocytes, which have shown encouraging outcomes in the management of chronic lymphoid leukaemia. Consequently, the FDA has granted approval to many therapeutic approaches using CAR-T cells for the treatment of lymphoma [100]. Although liquid malignancies have been effectively treated, significant challenges remain in using CAR-T cells to target solid tumors. The tumour immune-suppressive milieu is a key factor that hinders the activation of CAR-T cells in this context. When comparing liquid malignancies with dispersed tumour cells to solid tumors, it is seen that solid tumors have a more compact structure and greater pressure between tumour cells. This presents considerable obstacles for the penetration of CAR-T cells. In addition, many types of immune-suppressive cells present in the tumour microenvironment have the ability to hinder the function of CAR-T cells, even when they manage to penetrate the tumour tissue. Hence, the crucial aspect in both preclinical and clinical research efforts is in promoting the activity of CAR-T cells by modulating the tumour microenvironment [101].

5.2.2. Dendritic Cells

Dendritic cells (DCs) are the primary antigen-presenting cells (APCs) in the immune system. They play a crucial role in starting, regulating, and sustaining the immune response. This is due to their exceptional capacity to effectively collect, process, and display antigens. Immature dendritic cells (DCs) possess robust migratory capacities, but mature DCs are very proficient in activating naïve T cells. DCs in immunotherapy serve many functions: Dendritic cells (DCs) have the ability to express major histocompatibility complex (MHC) antigen molecules, collect tumour antigens, and then deliver them to T lymphocytes. 2) Once they reach maturity, dendritic cells (DCs) will exhibit elevated amounts of costimulatory molecules, including CD80, CD86, and CD40, in order to facilitate the activation of T cells and stimulate an immunological response [102]. Dendritic cells (DCs) have the potential to release cytokines, namely IL-12, as well as chemokines. This secretion serves to facilitate the gathering of T cells and improve their long-lasting capacity to eliminate infections. Due to the potent antigen presentation and immune regulation capabilities of dendritic cells (DCs), there has been much development of DC-based delivery methods and vaccines [103].

5.2.3. Macrophages

Macrophages are a subset of phagocytic cells that originate from monocytes. Their physical structure undergoes alterations over various functional phases. Resting macrophages typically have a circular or elliptical form with short protrusions. However, when activated, they tend to extend longer pseudopods and exhibit unusual morphologies. Macrophages play an active role in the body's innate immune response by scavenging invading pathogens. Macrophages primarily perform the task of engulfing and digesting cell debris and pathogens, a process known as phagocytosis [104]. They do not exhibit significant selectivity towards the antigen phenotype. Furthermore, macrophages serve as antigen presenting cells, therefore stimulating the adaptive immune response by the presentation of antigens to T cells. Distinct phenotypes of macrophages may have varying effects on the regulation of the immune system, including conflicting antitumor immune responses. Tumour-associated macrophages (TAMs) of the M1 phenotype has the ability to impede tumour development, whilst TAMs of the M2 phenotype have the capacity to facilitate tumour growth. Hence, decreasing M2 macrophages or stimulating the transformation of M2 macrophages into M1 macrophages are efficient approaches to enhance the immunosuppressive microenvironment of solid tumours. Inflammation is intricately linked to the facilitation and progression of tumours, whereby macrophages may detect chemotactic signals and relocate to the site of inflammation. The capacity of macrophages to specifically target tumours makes them very promising for delivering drugs [105].

5.2.4. Natural Killer Cells

Natural Killer (NK) cells constitute around 5-10% of lymphocytes in circulation. Natural Killer (NK) cells has the ability to eliminate infections and tumour cells *via* a non-specific mechanism, independent of antigen presentation. Natural Killer (NK) cells release cytotoxic substances such perforin and tumour necrosis factor (TNF) in order to eliminate target cells. In addition, they play a role in hypersensitivity responses and auto-immune disorders, whereby NK cells become excessively active and target healthy tissues. Within

the tumour microenvironment, natural killer (NK) cells often experience a decline in their functional abilities as a result of the suppression of activated receptors and the enhancement of inhibitory receptors. In this context, the application of cytokines and antibodies may often be used to stimulate their immunological activation [106].

5.2.5. Personalized Immunomodulation

Customizing immunomodulatory tactics according to a person's distinct immunological profile is known as personalized immunomodulation, a rapidly developing idea in the field of gene therapy. This strategy aims to maximize the safety and effectiveness of gene therapy interventions by tailoring immunomodulation to the unique features of each patient's immune system, taking into account the inherent heterogeneity in immune responses among individuals. Technological developments in genomes, transcriptomics, and proteomics have made it possible to gain a deeper understanding of each person's unique immune response [107]. Through the examination of genetic markers, gene expression patterns, and protein profiles, scientists can discern differences in immune function that could potentially influence the outcome of gene therapy. Based on this data, customized immunomodulation plans that cater to the unique needs and features of every patient's immune system can be created. Personalized immunomodulation is modifying immunomodulatory drug dosage and timing in response to unique patient responses. By tailoring, the immune system's overall integrity is to be preserved while immune responses against transgenes and therapeutic vectors are suppressed to the best possible extent. Additionally, more accurate decisions about the planning and execution of gene therapy are made when one is aware of a patient's pre-existing sensitivity to viral vectors or certain genetic variables impacting immune responses [108]. Personalized immunomodulation in cancer gene therapy may entail locating certain tumour antigens or neoantigens particular to a patient's cancer cells. The development of tactics to improve the immune system's capacity to identify and target cancer cells can be guided by this knowledge, which will ultimately increase the effectiveness of treatment. Although personalized immunomodulation is still a relatively new idea, continuous research and technology developments are opening the door to more individualized and patient-specific gene therapy techniques. The ultimate objective is to optimize the advantages of gene therapy while reducing any possible hazards, keeping in mind the variations in immune responses seen in different patient groups [109].

5.2.6. Nanoparticle-based Delivery Systems

The presence of antigens and adjuvants is crucial for effectively stimulating the immune function. However, their limited safety and effectiveness hinder their utilisation in clinical research. Nanotechnology offers the potential to create a new category of antimicrobial drugs. In recent times, there has been a significant focus on virosome and liposome-derived nanovaccines that specifically target viral diseases. These vaccines have gained considerable attention due to their exceptional effectiveness within the body. The integration of immunotherapy with nanomedicine can effectively overcome the limitations of therapeutic results and enhance the bioactivity of immunotherapeutic agents [110]. Immunotherapy commonly utilises substances that have the ability to either stimulate or inhibit the immune system. Immunostimulatory drugs are employed in antiviral therapy to enhance the immune response to viruses by recognising and eliminating foreign antigens, as well as generating immune cells with memory capabilities for viral infections. Immunomodulatory nanosystems are created by coating or conjugating with fragments of antigens and adjuvants in order to augment the functionality of the immune system, as antigens alone are susceptible to destruction by enzymes within the body. Linking adjuvants can prevent the degradation of antigens and enhance the activation of antigen-presenting cells (APCs) more efficiently. Immunomodulatory nanoparticles have the ability to stabilise antigen-adjuvant complexes in the bloodstream and facilitate the controlled release of antigens to specific locations. The gradual release of the nanosystem can hinder the excessive activation of the immune system by intercepting the undesired immune response and eliminating the foreign molecules. The manipulation of multiple crucial components of the human immune system by different nanoformulations leads to the regression of viral infections [111]. Nanoparticles augment the immunomodulatory impact of enclosed payloads. Nano-delivery methods offer distinct benefits in transporting therapeutic payload to regulate immune cell function. Dendritic cell (DC) targeted nanoparticles that might elicit immunological tolerance are categorised into four primary groups based on the therapeutic cargo they transport. 1) Nanoparticles loaded with peptides linked to autoantigens stimulate the generation of T cells that target specific antigens. 2) Nanoparticles containing immunomodulatory medicines that promote the transformation of immature dendritic cells into tolerant DCs. 3) Nanoparticles containing nucleic acids or plasmids that possess gene editing properties, which inhibit the co-stimulatory signalling pathway between dendritic cells and T cells. 4) Nanoparticles co-deliver autoantigen-associated peptides, immunomodulatory medicines, and nucleic acids. In this discussion, we will briefly explore the function of therapeutic cargo-targeted dendritic cells (DCs) following nano-delivery in the management of autoimmune disorders, allergy diseases, and transplant rejection diseases [112].

5.3. Gene Editing Technologies

Gene editing introduces a new target for the immune system: the response to the proteins utilised for double-strand DNA breakage. While significant progress has been made in comprehending the mechanisms of B-cell response to vectors that transport CRISPR/Cas9 into cells, the T-cell response has only been recently demonstrated. In a study conducted by Wang *et al.* (2015), it was discovered that immunocompetent mice exhibited a T-cell response to Cas9 proteins. A Streptococcus pyogenes-derived Cas9 system, supplied by an adenovirus vector, was used to target the Pten gene. This gene is often altered in patients with sporadic cancer and is also involved in nonalcoholic steatohep-

atitis (NASH) [113]. The study demonstrated that hepatocytes were depleted due to both humoral and cellular immune responses against the AdV vectors, occurring within a timeframe of two weeks to four months following the injection. However, they also observed an immunological reaction to the Cas9 protein, which was identified using an ELISA technique to detect SpCas9 antibodies. Furthermore, a strong production of IgG1 antibodies against Cas9 was seen fourteen days following the delivery of the adenovirus. Chew *et al.* (2016) and Chew (2018) have demonstrated a similar reaction whether use AAV or electroporation techniques to excessively express a transgene in the identical species of animal. In 2016, the researchers evaluated the efficacy of AAV-Cas9-gRNA targeting Mstn (AAV9-Cas9-gRNAM3+M4) by administering it through intraperitoneal injection in neonatal mice [114]. The ELISA experiment has verified the presence of a specific immune response against Cas9, and the Cas9 peptides were identified using serum from the animals by utilising M13 phage libraries that contained the Cas9 transgene. A study by Ajina *et al.* (2019) discovered the presence of T-cell response in tumours that were transplanted into mice with a normal immune system and had an increased expression of SpCas9. Furthermore, Li *et al.* (2020) showed that administering SaCas9 immunisations to mice one week before to AVV-liver treatment delivery resulted in reduced long-term survival of the *in vivo* altered hepatocytes. This indicates that the immune system may react to treatments involving Cas9 proteins as a result of the immune system's ability to remember previous infections [115]. Crudele and Chamberlain (2018) provided insight into a pre-existing immune response to two prevalent human infections, Staphylococcus aureus and Streptococcus pyogenes, which are responsible for producing MRSA and strep throat, respectively. These pathogens are the source of the Cas9 protein. In a study conducted by Charlesworth *et al.* (2019), it was demonstrated that our immune system has the ability to identify Cas9 peptides as foreign substances. The research also revealed that among healthy adult humans, the occurrence of an immune response against Cas9 is 79% for SaCas9 and 67% for SpCas9, as indicated by the presence of anti-Cas9 IgG antibodies. Simhadri *et al.* (2018) reported prevalence rates of approximately 10% and 2.5% for anti-SaCas9 and anti-SpCas9, respectively, in samples collected from the United States [116]. Studies conducted by Wagner *et al.* (2019) and Ferdosi *et al.* (2019) have yielded comparable findings, indicating that 85% and 5% of blood donors possess anti-SpCas9 antibodies and anti-SpCas9 T cells, respectively. Charlesworth and Wagner have demonstrated a similar reaction by utilising the complete recombinant protein in their experiments, employing ELISPOT and flow cytometry techniques. However, Ferdosi *et al.* (2019) adopted a distinct methodology. By utilising computational methods, a group of researchers chose and constructed a collection of 38 peptides for experimentation. These peptides were then tested using HLA-A*02:01 pentamers, which were able to detect immune responses through ELISPOT and flow cytometry techniques. The results showed that 83% (n=12) of the samples had a positive reaction, as shown by the presence of IFN-γ. Stadtmauer *et al.* (2020) employed an identical set of peptides and proposed that 66% of the sample (n=3) exhibited a response to SpCas9. Furthermore, they documented the initial human clinical experiment specifically aimed at evaluating the safety and practicability of CRISPR-Cas9 modification of T cell receptors [117].

6. ADVANCED MONITORING TECHNIQUES

In the field of gene therapy, advanced monitoring tools are essential because they give researchers and doctors a comprehensive understanding of immune response dynamics, therapeutic efficacy, and possible side effects. Throughout the course of treatment, these monitoring techniques aid in the development of a thorough understanding of the interactions between the host immune system and the therapeutic interventions, enabling informed decision-making and real--time modifications. The adoption of advanced imaging technology is one of the noteworthy developments in monitoring techniques [118]. With the use of non-invasive imaging techniques like magnetic resonance imaging (MRI) and positron emission tomography (PET), scientists can monitor the distribution and durability of gene therapy vectors in the body. This aids in tracking the targeting precision of therapeutic drugs, evaluating their biodistribution, and locating any possible off-target effects. Apart from imaging, sophisticated molecular and cellular monitoring methods offer information at the cellular and genetic levels. Researchers can monitor the overall effect of gene therapy on the host transcriptome, assess gene expression profiles, and spot changes in immune cell populations thanks to high-throughput sequencing technology. The characterisation of immune responses is further improved by flow cytometry and single-cell analysis techniques, which offer comprehensive details about particular cell types and their activation status [119].

Finding and analysing biomarkers is another facet of sophisticated monitoring methods. Through the identification and measurement of particular biomarkers linked to immune responses or therapeutic efficacy, researchers can create prediction indications for treatment results. This makes it easier to create individualized treatment plans based on the unique characteristics of each patient. Ongoing developments in the fields of omics technologies, including as transcriptomics, proteomics, metabolomics, and genomics, add to our understanding of the complex interactions that take place during gene therapy [120]. These methods provide a comprehensive picture of the molecular terrain, illuminating putative mechanisms of action, immunological regulation, and the maturation of immune memory. As gene therapy develops, it will be crucial to incorporate cutting-edge monitoring methods into clinical trials to maximize therapeutic approaches and guarantee patient security. A more sophisticated and knowledgeable approach to gene therapy is made possible by the integration of imaging, molecular profiling, and biomarker analysis, which eventually opens the door to safer, more efficient, and customized therapies [121].

7. CASE STUDIES

7.1. Case Study 1: AAV-Mediated Gene Therapy for Hemophilia A

In this case, researchers explored the use of adeno-associated virus (AAV) vectors for delivering the clotting factor VIII gene to patients with hemophilia A. While initial trials showed promising results, the immune response against the AAV vector posed a challenge. To overcome this, the team employed immune modulation strategies, including co-administration of immunosuppressive agents. This approach successfully mitigated the immune barriers, allowing sustained expression of the therapeutic gene and significantly enhancing the efficacy of the gene therapy in hemophilia A patients [122].

7.2. Case Study 2: CRISPR-Cas9 Gene Editing for Duchenne Muscular Dystrophy (DMD)

Scientists aimed to address immune challenges associated with CRISPR-Cas9 gene editing in treating Duchenne Muscular Dystrophy. While the precision of CRISPR-Cas9 in correcting the dystrophin gene mutations was remarkable, the immune response triggered by the edited cells raised concerns. To optimize the therapy, the research team integrated immune-modulating nanoparticles that selectively dampened the immune response without compromising the editing efficiency. This innovative approach successfully overcame immune barriers, allowing for sustained dystrophin expression and improved muscle function in preclinical models [123].

7.3. Case Study 3: CAR-T Cell Therapy for Solid Tumours

Investigating immune modulation in the context of CAR-T cell therapy for solid tumours, researchers faced challenges due to the hostile tumour microenvironment. The immune-suppressive nature of the tumour hindered CAR-T cell persistence and efficacy. In response, scientists developed a combination approach using gene editing techniques to enhance CAR-T cell resistance to immunosuppression, coupled with local administration of immune checkpoint inhibitors. This integrated strategy effectively overcame the immune barriers within the tumour microenvironment, leading to improved infiltration, persistence, and antitumor activity of CAR-T cells in experimental models [124].

8. CHALLENGES AND OPPORTUNITIES

Research on immune modulation techniques in gene therapy is a dynamic and quickly developing topic that offers both potential and problems. Overcoming the human immune response to the therapeutic gene delivery vectors is one of the main obstacles. The immune system frequently quickly eliminates these vectors because it views them as foreign invaders, which reduces the effectiveness of gene therapy. This immunological identification may set off inflammatory reactions, which might have negative consequences and lessen the treatment's therapeutic effectiveness. Furthermore, a patient's pre-existing immunity from prior exposure to viral vectors or other gene therapy components may make the treatment more difficult to achieve. However, these difficulties also provide fascinating chances for advancement and creativity [125]. Scholars are investigating several approaches to regulate the immune system and reduce its reaction to gene therapy vectors. To prolong the presence of therapeutic drugs in the body and avoid immune detection, for example, researchers are developing immune-evasive or stealth vectors. It is also possible to make gene therapy safer and more effective by making engineering vectors less immunogenic and better at targeting specific tissues. Innovative methods, such as encasing therapeutic genes in nanoparticles or biomaterials, have the potential to protect them from immune monitoring and enable targeted distribution. Moreover, knowing the subtleties of how the immune system reacts to gene therapy provides opportunities for customised therapeutic approaches. Customising therapies according to a person's immunological profile may reduce the likelihood of immunological responses and improve treatment results [126]. Research on immunology and gene therapy may work together to find novel ways to modulate the immune system, which might lead to the creation of safer and more efficient gene treatments. The continuing investigation of immune modulation techniques offers a rich field for scientific learning and the achievement of gene therapy's maximum potential in therapeutic applications as we traverse these difficulties. Researchers may customise gene therapy therapies to minimise the danger of immune responses and maximise therapeutic advantages by knowing each patient's unique immunological profile. Immunologists and gene therapy specialists working together might transform the industry and lead to safer and more effective gene treatments. By investigating immune modulation techniques further, we are opening up new avenues for scientific discovery and laying the groundwork for gene therapy's broad use in a range of therapeutic settings [127].

9. ETHICAL CONSIDERATIONS

We will also discuss the significance of personalized medicine in tailoring gene therapy approaches to the individual immunological profile of each patient to maximize therapeutic benefits and minimize adverse events. This work aims to provide a critical synthesis of the current state of immune modulation strategies, offering insights into the challenges faced by medical professionals and researchers as the area of gene therapy develops swiftly. Gaining an understanding of the intricate relationship between gene treatments and the immune system would enable the development of safer, more effective, and widely applicable gene-based medications for a variety of illnesses [128]. Ultimately, this knowledge will improve many people's lives and revolutionise the medical sector. In the end, by revolutionising treatment choices and increasing patient outcomes, this knowledge will improve countless lives and revolutionise the medical profession. Furthermore, by comprehending how gene therapies interact with the immune system, researchers may better handle the possible negative responses

and side effects that may result from these treatments. Researchers may create techniques to reduce immune rejection and maximise the efficacy of gene-based medications by deciphering the complex interaction between gene treatments and the immune response [129]. This information will not only lead to more effective gene therapy treatments, but it will also provide opportunities for cutting-edge targeted therapy and personalised medical strategies. In the end, these developments might transform healthcare and provide hope to those afflicted with illnesses that are presently incurable. The ability to personalize gene treatments for particular patients might greatly improve medicine in the future. Scientists can create medicines that the body is less likely to reject by knowing how the immune system reacts to these treatments. In addition to raising the likelihood of a successful course of therapy, this personalized approach creates new opportunities for targeted medicines, which include targeting individual genes to cure a given illness. Gene therapy has the potential to revolutionize healthcare and provide hope to those who have been declared incurable with further developments [130].

10. FUTURE DIRECTIONS

A number of directions need investigation as we dive further into the intriguing world of immune modulation techniques in gene therapy in order to advance the discipline and optimise therapeutic effectiveness. The development of patient-specific precision medicine strategies is one such avenue. By using the potential of personalised gene therapy, we might imagine the creation of treatments that target certain genetic differences in patients as well as immune response modulation. Reducing off-target effects and improving treatment results, this may open the door for more focused and successful approaches. In addition, there is a great deal of promise in combining cutting-edge technologies like CRISPR/Cas9 and other genome-editing instruments. Subsequent investigations have to concentrate on improving these instruments to augment their safety and specificity, permitting accurate editing of genes linked to the immune system [131]. This may make it possible to fine-tune immune responses, which would eventually result in a more regulated and controlled treatment outcome. It also seems that investigating combinatorial methods is a viable avenue for the future. Immune modulators may have synergistic effects when used with conventional gene therapy vectors or other treatment methods, increasing their total therapeutic effectiveness. Finding the best mixtures and doses should be the focus of research, as should comprehending the intricate interactions between the many elements in these diverse strategies. Furthermore, a vital frontier is the development of delivery technology. Enhancing the delivery of gene treatments to certain cells or tissues may reduce systemic effects and improve overall safety profiles. By creating more efficient and customized delivery methods, this is possible [132]. For example, nanotechnology shows potential here, and more study should focus on improving and developing delivery systems. It is crucial that we comprehend the long-term consequences of immune regulation techniques as we go forward. It is critical to conduct comprehensive investigations on the duration of altered immune responses in order to ensure that gene treatments are both safe and effective over extended periods of time. When combined with empirical data, longitudinal studies will provide important new information on the long-term viability and any adverse impacts of these novel strategies. Finally, it should be noted that immune modulation techniques for gene therapy have a dynamic and multifaceted future [133]. The future roadmap includes precision medicine, sophisticated genome-editing tools, combinatorial methods, improved delivery systems, and long-term safety evaluations. We can fully realize the promise of immune regulation in gene therapy by tackling these obstacles and pushing the frontiers of scientific discovery, ushering in a new age of individualised and efficient therapies for a wide range of illnesses. These developments have potential applications in the treatment of infectious illnesses, autoimmune diseases, cancer, and genetic abnormalities. For example, precision medicine enables individualised therapy based on a patient's genetic composition, optimising treatment effectiveness and reducing adverse effects. With the capacity to precisely alter genes, cutting-edge genome-editing technologies like CRISPR-Cas9 provide new avenues for targeted gene therapy [134]. Combinatorial methods, which integrate many immune modulation techniques, may improve treatment results by focusing on several immune system components in concert. Viral vectors and nanoparticles are examples of sophisticated delivery technologies that guarantee the effective and precise transfer of therapeutic genes to the intended cells or tissues. Finally, to guarantee the longevity and security of gene therapy treatments, long-term safety evaluations are essential. Immune regulation in gene therapy is positioned to transform medicine and enhance the lives of many people by tackling these obstacles and using these cutting-edge strategies [135].

CONCLUSION

It is crucial to continue researching immune modulation techniques in gene therapy since they may be able to overcome immune system obstacles and improve the overall efficacy of these novel therapies. The complex interaction between gene treatments and the human immune system has long presented difficulties, preventing gene therapies from reaching their full potential. On the other hand, a thorough comprehension and use of immune modulation strategies provide viable remedies. Gene therapy may be tuned to minimise side effects and optimise therapeutic benefits by adjusting the immune response. Immunosuppressive medications, for example, may lessen the immune system's reaction to the viral vectors used in gene therapy, lowering the possibility of rejection and inflammation. Additionally, developments in gene editing technologies like CRISPR make precise adjustments to immune cells themselves possible, paving the way for the creation of customized medicines that can evade immune recognition and destruction. The area of gene therapy might undergo a revolution and patient outcomes could be greatly improved by further investigation into immune regulation techniques. The wide range of approaches cov-

ered, from tailored delivery systems to immunosuppressive medications, highlights how active this area of study is. To maximise the safety and efficacy of gene treatments, researchers work to achieve a finely balanced response from the immune system while avoiding harmful side effects. Moreover, the identification of unique immune profiles and the creation of tailored strategies demonstrate a paradigm shift in gene therapy towards precision medicine. There is great potential to minimise side effects and maximise therapeutic success by customising therapies to each patient's unique immunological environment. The incorporation of strong immune modulation techniques will surely be crucial in determining the outcome of these ground-breaking gene therapy therapies as we go forward. To improve current methods, find fresh strategies, and deal with new issues, immunologists, geneticists, and physicians must continue to collaborate. Gene treatments might one day reach their full potential thanks to the unwavering search for novel approaches to immune regulation, providing hope to those suffering from a wide range of hereditary illnesses and disorders. The possibility of breaking down immunological barriers and improving gene therapy's overall effectiveness as a result of these coordinated efforts presents an intriguing and revolutionary new frontier in the field of medical research. Since each group brings a different set of skills and views to the table, collaboration between immunologists, geneticists, and physicians is essential. Together, they may create new strategies, enhance existing methods, and address the changing issues in the area of gene therapy. This collaborative endeavour gives hope to those suffering from a variety of genetic illnesses and disorders and presents prospects for gene treatments to reach their full potential. As a result, it holds significant promise for the future. This promising and revolutionary area of medicine has the potential to improve gene therapy's overall efficacy and break down immunological barriers, which would eventually benefit a great number of people.

AUTHORS' CONTRIBUTIONS

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

LIST OF ABBREVIATIONS

AAV = Adeno-associated Virus

ALRs = AIM2-like Receptors

APCs = Antigen-presenting Cells

CLRs = C-type Lectin Receptors

DCs = Dendritic Cells

DIC = Disseminated Intravascular Coagulation

HSCT = Hematopoietic Stem Cell Transplantation

MHC = Major Histocompatibility Complex

MRI = Magnetic Resonance Imaging

mTOR = mammalian Target of Rapamycin

NASH = Nonalcoholic Steatohepatitis

NF-κB = Nuclear Factor κB

NK = Natural Killer

NLRs = Nod-like Receptors

PET = Positron Emission Tomography

PRRs = Pattern Recognition Receptors

RLRs = RIG-I-like Receptors

SOT = Solid Organ Transplantation

TAMs = Tumour-associated Macrophages

TLRs = Toll-like Receptors

TNF = Tumour Necrosis Factor

vWF = von Willebrand Factor

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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