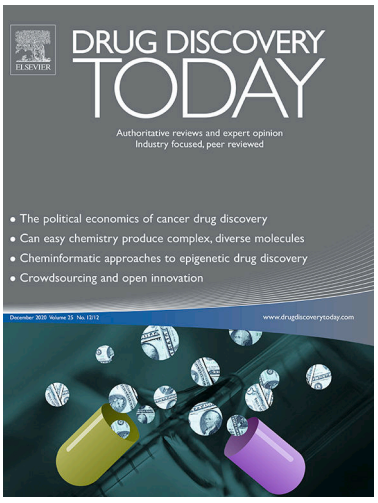
Journal Pre-proofs



Gene-to-screen (blue)

Decoys as potential therapeutic tools for diabetes

Maryam Mahjoubin-Tehran, Samaneh Rezaei, Stephen L. Atkin, Fabrizio Montecucco, Amirhossein Sahebkar

PII: S1359-6446(21)00191-4
DOI: https://doi.org/10.1016/j.drudis.2021.04.004
Reference: DRUDIS 2989

To appear in: *Drug Discovery Today*

Received Date: 7 November 2020
Revised Date: 15 January 2021
Accepted Date: 6 April 2021

Please cite this article as: M. Mahjoubin-Tehran, S. Rezaei, S.L. Atkin, F. Montecucco, A. Sahebkar, Decoys as potential therapeutic tools for diabetes, *Drug Discovery Today* (2021), doi: https://doi.org/10.1016/j.drudis.2021.04.004

Decoys as potential therapeutic tools for diabetes

Maryam Mahjoubin-Tehran[1,2†], Samaneh Rezaei[1,2†], Stephen L. Atkin[3], Fabrizio Montecucco[4,5] and Amirhossein Sahebkar[6,7,8,9*]

[1]Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Department of Medical Biotechnology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
[3]Weill Cornell Medicine Qatar, Doha, Qatar
[4]IRCCS Ospedale Policlinico San Martino Genoa – Italian Cardiovascular Network, 10 Largo Benzi, 16132 Genoa, Italy
[5]First Clinic of Internal Medicine, Department of Internal Medicine, University of Genoa, 9 viale Benedetto XV, 16132 Genoa, Italy
[6]Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[7]Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[8]Polish Mother's Memorial Hospital Research Institute (PMMHRI), Lodz, Poland
[9]School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[†]Equal contributions as first authors
Corresponding author: Sahebkar, A. (sahebkara@mums.ac.ir)

Teaser: This review discusses the application of decoy technologies, including decoy oligodeoxynucleotides and decoy peptides, as a promising therapeutic approach for diabetes.

Key words: decoy ODN, decoy peptides, diabetes, type 2 diabetes mellitus

Highlights:

- There is an urgent need for novel therapeutic approaches against diabetes.
- Decoy-based therapy is an emerging strategy that shows promise for diabetes and its complications.
- Decoy technologies include decoy oligodeoxynucleotides and decoy peptides.
- This review summarizes the therapeutic effects of decoy-based therapies in diabetes.

Current therapeutic approaches for diabetes are focused on improving glycemic control to prevent diabetes-related complications, but such approached are not completely successful. Decoy technologies such as decoy oligodeoxynucleotides (ODNs) and decoy peptides have emerged as therapeutic tools in diabetes. Decoy ODNs carry a DNA recognition motif for the binding of transcription factors in order to trap them and block their effects, whereas decoy peptides mimic the binding structure of the receptor protein, bind to the docking site of the target ligand, and prevent the interaction of the ligand and receptor. This review summarizes the technologies that have been developed to date and the studies that have investigated the therapeutic effects of decoy ODNs and peptides in diabetes.

Introduction

Diabetes mellitus (DM) is a major public health challenge in both developed and developing countries [1,2]. Diabetes is associated with both microvascular and macrovascular complications that result in considerable morbidity, escalating healthcare costs and death [3]. Various risk factors may increase the chance of developing diabetes. Among US adults with diagnosed diabetes (2013–2016) the most common risk factors were obesity, physical inactivity, hypertension, hypercholesterolemia and smoking [4]. Controlling for these factors is a major strategy for the prevention of diabetes-related complications [4]. Despite conventional therapies and recent advances in pharmacotherapy for DM, many patients are still unable to achieve optimal glycemic control and the burden of disease remains alarmingly high. Hence, there is a need to develop novel effective therapeutic strategies to control DM and to prevent its life-threatening complications.

Decoys as a novel therapeutic approach

Recently, decoy technology has been proposed as an effective therapeutic tool against a variety of diseases such as different types of cancer [5–10], atherosclerosis [11], heart failure [12], abdominal aortic aneurysm [13] and COVID-19 [14]. Decoy technology mainly encompasses the use of decoy oligodeoxynucleotides (ODNs) and decoy peptides (**Figure 1**). The regulation of gene expression by transcription factors plays crucial roles in the development of various diseases; therefore, transcription factors are considered as potential therapeutic targets [15]. Decoy ODNs are short double-stranded DNA that bind to the transcription factors and in this way they block the expression of specific genes at the transcriptional level [16]. Decoy ODNs must carry the optimal DNA recognition motif for a regulatory site in order to trap transcription factors and block their effects [16–18]. Many recent studies have aimed to improve the pharmacological characteristics and chemical modifications of ODNs in order to increase their biostability, resistance to serum nucleases, and cell and nuclear adsorption **{AuQ: Edit OK?}** [19].

Decoy peptides are relatively short amino acid sequences that mimic the binding structure of the receptor protein. Such a peptide could retain the ability to recognize and bind the docking site of the target ligand, thereby preventing the interaction of the ligand and receptor and blocking the corresponding signal [20].

The application of decoy technologies as therapeutic tools has been also considered in diabetes. In this review, we summarize studies that have investigated the effects of ODN and peptide decoy in diabetes (**Table 1**).

Targets of decoys in diabetes

Fas–Fas ligand

Fas ligand (FasL) is a type 2 cell membrane protein belonging to the tumor necrosis factor (TNF) family that binds to its receptor Fas, thus mediating the apoptosis of Fas-expressing cells. Impairment of the Fas system causes lymphoproliferative disorders and accelerates

autoimmune diseases, whereas its amplification may result in tissue destruction [21]. Accordingly, autoimmune diabetes is caused by beta cell destruction by islet-reactive T cells, a process related to beta cell apoptosis (**Figure 2**). Both Fas and FasL are expressed by beta cells in response to cytokine stimulation. Moreover, transgenic non-obese diabetic (NOD) mice whose beta cells express a FasL transgene developed an accelerated form of diabetes. Thus, interventions that inhibit the Fas–FasL pathway could be promising for the treatment or prevention of type 1 diabetes [22].

According to Kawamoto *et al.* [23], pig islets are the most appropriate origin of islets for xenotransplantation into patients suffering from type 1 diabetes mellitus. However, the long-term survival of xenografts is hampered by cellular rejection and, in particular, by CD8 cytotoxic T lymphocyte (CTL)-mediated cytotoxicity. These authors also demonstrated that potent human CTL-mediated cytotoxicity was promoted by the Fas–FasL apoptotic pathway. To address this, they employed a novel approach in which human CD8 CTL-mediated xenocytotoxicity was inhibited by upregulation of a human decoy Fas antigen and membrane-bound human FasL in xenografted cells, seeking to determine whether these inhibitory molecules afforded cytoprotective effects. They transfected the isolated pig islets with adenovirus vectors that encoded either membrane-bound human FasL or human decoy Fas genes: almost 60% of transfected islets expressed the encoded molecules. In comparison with parental pig islets, these molecules showed 60–88% suppression of CTL-mediated cell death **{AuQ: Edit OK?}**. These results demonstrated that isolated pig islets from transgenic pigs expressing either of the mentioned above genes might determine the innate cellular response to xenografts. Therefore, this method creates a new opportunity to improve xenograft survival [23].

Heme oxygenase 1 (HO-1) has been shown to have an antioxidative, antiapoptotic, and anti-inflammatory effect and can protect cells from immune system attack. It has been shown that decoy receptor 3 (DcR3) can block Fas-ligand-induced pancreatic cell injury in autoimmune diabetes. Furthermore, Huang *et al.* [24] showed that overexpression of murine HO-1 (mHO-1) and DcR3 in transgenic islets extended their survival time compared with that of nontransgenic islets. DcR3 and mHO-1 double-transgenic islets of NOD mice, however, had less protection against cytotoxic agents and shorter islet graft survival period than single-transgenic DcR3 or mHO-1 islets **{AuQ: Edit OK?}** [24].

Wang *et al.* [25] reported that the transgenic expression of DcR3 in a cell-specific manner offered remarkable protection from autoimmune diabetes to NOD mice. DcR3 is a member of the TNF receptor superfamily that modulates immune responses by competing with receptors of TNF-like molecule 1A (TL1A), LIGHT and FasL. These investigators explored the systemic effect of DcR3 in modulating dendritic cells and lymphocytes in NOD mice. Their results indicated that both DcR3 protein and DcR3-encoding plasmids **{AuQ: Edit OK?}** could substantially inhibit diabetes and insulitis. Mice that were treated with the DcR3 peptide fused to the mouse Fc region (DcR3.Fc) showed lower proliferation of lymphocytes and ameliorated diabetes **{AuQ: Edit OK?}**. Double transgenic NOD mice, which expressed DcR3.Fc and either the human Thy1 cell surface marker under IFN-promoter control or the

murine Thy1.1 cell surface marker under interleukin (IL)-4 promoter control, demonstrated a significant decrease of Th1-mediated and increased Th2-mediated immune responses *in vivo* **{AuQ: Edit OK?}**. In addition, *in vitro* polarization experiments indicated that the differentiation of both Th1 and Th17 cells was mainly prevented when the DcR3.Fc protein was enhanced in **{AuQ: Edit OK?}** splenocytes. However, this was not seen in purified CD4+ T cells. Therefore, it seems that DcR3-induced inhibition of Th1 and Th17 differentiation was not T-cell-autonomous and might be mediated by other cell types such as dendritic cells. Wang *et al.* [25] concluded that DcR3 can directly regulate the maturation and differentiation of dendritic cells and subsequently modulates the effector function and differentiation of T cells.

In 2004, Wu *et al*. [26] used bone-marrow-derived dendritic cells (BM-DCs) obtained from NOD mice cultured with recombinant DcR3.Fc protein to study the modulatory effects of DcR3 on the function and differentiation of DCs. In addition, the T-cell-stimulating functions and differentiating phenotypes of these DCs **{AuQ: Edit OK?}** were investigated. Reduced expression of MHCI- Ag7, CD40, CD54, CD11c was observed in DCs incubated with additional DcR3.Fc rather than with granulocyte macrophage-colony stimulating factor (GM-CFS) or IL-4 **{AuQ: Edit OK?}**. Their results demonstrated that DcR3 restricts the maturation and differentiation of BM-DCs. The most significant effects of DcR3.Fc on the differentiation of DCs were the downregulation of CD80 and the upregulation of CD86, indicating that DcR3.Fc has the regulatory potential to change the T cell response to the T helper cell type 2 (Th2) phenotype. Accordingly, the proliferation of CD4 T cells that are co-cultured with DcR3.Fc-treated DCs is significantly less than that of T cells stimulated by normal DCs **{AuQ: Edit OK?}**. The secretion of interferon by T cells that were co-cultured with DcR3.Fc-treated DCs was also suppressed, showing that DcR3 has a Th1-suppressing effect on the differentiation of DCs. Adoptive transfer experiments also showed that NOD or severe combined immunodeficiency mice that received DcR3.Fc-treated DCs and then autoreactive T cells demonstrated delayed initiation of diabetes and a reduction in diabetes severity, when compared with mice that received normal DCs and T cells. These results suggested that this treatment may have clinical utility for future therapeutic use in autoimmune diabetes. MALDI-TOF and 2D gel electrophoresis data from DcR3-treated cells illustrate the overexpression of some proteins (cyclin-dependent kinase 6, mitogen-activated protein kinase p38, and signal-induced proliferation-associated gene 1) and reduced expression **{AuQ: Edit OK?}** of the TNF-related apoptosis-inducing ligand family member-associated NFkB activator-binding kinase 1, the IL-17 precursor, and Golgi *S*-nitroso-*N*-acetylpenicillamine, further suggesting that DcR3 affects the differentiation and function of DCs [26].

Scarcity of human donors is a major restriction for clinical islet transplantation for the treatment of patients with type 1 diabetes. Pig islet xenotransplantation seems to be an unlimited source of donor pancreas, but Kawamoto *et al.* [27] showed that cell-mediated rejection, particularly as a result of human CD8 CTL-induced cytotoxicity, was the main barrier for long-term survival of islet xenografts. These researchers also showed that upregulation of either human decoy Fas antigen (decoy Fas) or membrane-bound human FasL (mFasL) in pig

islets not only impeded CTL xenocytotoxicity *in vitro*, but also lengthened the histological survival of pig islet xenografts *in vivo*. Thus, they decided to investigate the potential beneficial effect of the adenoviral transfer of these genes into pig islets *ex vivo* before transplantation on the post-transplantation glycemic control of diabetic rats. To do this, isolated pig islets were transfected with an adenovirus vector containing complementary DNA (cDNA) of either decoy Fas or mFasL. Subsequently, the transfected islets were transplanted below the kidney capsule of diabetic rats. These rats demonstrated a remarkable reduction in the levels of blood glucose from 12–18 hours after transplantation when compared to control groups [27].

Another study investigated new approaches for preventing the overexpression of either membrane-bound human FasL or human decoy Fas antigen in pig endothelial cells. Isolated islets were transfected with an adenoviral expression vector containing the DNA fragments encoding these proteins **{AuQ: Edit OK?}**. Transfected islets were then transplanted into pre-immunized diabetic rats. This resulted in near 80% suppression of cytotoxicity in membrane-bound human FasL-expressing pig islets and 60% inhibition of CTL killing in decoy Fas expression pig islets in an *in vitro* assay. In an *in vivo* transplant model, Kawamoto *et al.* [28] observed extended survival of pig islet xenografts expressing either human decoy Fas genes or membrane-bound human FasL. They concluded that the extracellular remodeling of either death-ligand or death receptor genes could be effective for the prevention of CTL-mediated xenocytotoxicity in pig islets [28].

DCR3 prevents both LIGHT- and Fas-ligand-mediated cell death, which are key for pancreatic cell damage in autoimmune diabetes. Sung *et al.* [29] studied the therapeutic capability of DCR3 in halting this process. Using transgenic non-obese diabetic mice that were characterized by DCR3 overexpression in their cells, they showed that transgenic DCR3 could protect mice from autoimmune and cyclophosphamide-mediated diabetes in a dose-dependent manner and remarkably eliminated insulitis. Furthermore, local expression of the transgene did not change the diabetogenic attributes of systemic lymphocytes or the development of T regulatory cells or T helper 1. Sung *et al.* [29] also showed that the success rate of transplantation was higher in transgenic islets, which survived longer than wild-type islets. They showed for the first time that the immune-evasion function of DCR3 hampers autoimmunity and concluded that the genetic modification of grafts may increase the survival and success of islet transplants [29].

Nuclear factor-κB (NF-κB)

NF-κβ plays a key role in the development of DM and in diabetes-related complications, for example, in the pathogenesis of vascular complications. Chronic hyperglycemia activates NF-κβ, which triggers the expression of various pro-inflammatory cytokines, chemokines and cell adhesion molecules, and regulates both the survival and death of beta cells. Overexpression of TNF-α, interleukins, TGF-β, Bcl2 and other pro-inflammatory proteins and pro-apoptotic genes by NF-κβ is a major risk factor in vascular dysfunction. In type 1 diabetes (T1D), interleukin-1 β-induced NF-κB activation causes the apoptosis of beta cells in the pancreas.

However, in type 2 diabetes, activated NF-κB induces both apoptosis and insulin resistance. Reactive oxygen species (ROS) and advanced glycation end products (AGEs) contribute to the progression of both micro- and macro-complications of DM. There is evidence of the activation and involvement of NF-κB in all major diabetic complications, including diabetic cardiomyopathy, retinopathy, nephropathy, and neuropathy. Hence, inhibition of the NF-κβ pro-inflammatory pathway may be a promising novel target for the management of diabetes complications [30,31].

Quan *et al.* [32] suggested that certain risk factors could stimulate the initiation of insulin-dependent diabetes mellitus (IDDM). They showed that both the production of ROS and the activation of the transcription factor NF-κB had a strong connection with the induction of IDDM *in vivo*. To investigate the role of NF-κB activation in diabetogenesis, Quan *et al.* [32] used alloxan, a diabetogenic chemical, as a model for inducing diabetes through destruction of insulin-producing pancreatic beta cells. Alloxan injection in mice resulted in the rapid and specific activation of NF-κB in the pancreas. When NF-κB decoy oligodeoxynucleotides were administered before the alloxan, the activation of pancreatic NF-κB was effectively blocked in mice, pancreatic cell death was prevented, insulin secretion was restored, and hyperglycemia was reversed. As a control, administration of scrambled NF-κB decoy showed no effect on alloxan-induced diabetes in mice [32].

Zhong *et al.* [33] explored how NF-κB contributes to insulin resistance in T2DM. They obtained subcutaneous abdominal adipose tissue from T2DM patients and non-diabetic control subjects. Pre-adipocyte cultures were differentiated into adipocytes *in vitro.* Immunoblotting and immunoprecipitation were used to examine AKT (Ser473) phosphorylation and IRS-1 tyrosine levels after insulin stimulation. IL-6 and MCP-1 levels, as well as the DNA-binding activity of NF-κB, were studied by enzyme-linked immunosorbent assay (ELISA) and electrophoretic mobility shift assay (EMSA). To evaluate molecular events, NF-κB decoy molecules were introduced into T2DM adipocytes. The study demonstrated that, in response to insulin stimulation, adipocytes from T2DM patients showed insulin resistance along with a marked decreases in the levels of AKT (Ser 473) phosphorylation and of IRS-1 tyrosine in comparison with those in cells from non-diabetic subjects. T2DM cells exhibited high levels of MCP-1 and IL-6 and of NF-κB activity. Administration of a NF-κB decoy decreased both IL-6 secretion and NF-κB activity, while increasing AKT (Ser473) phosphorylation and insulin-stimulated IRS-1 tyrosine in T2DM adipocytes. Zhong *et al.* [33] concluded that abdominal subcutaneous fat cells derived from T2DM patients showed micro-inflammatory and insulin resistance status, and that NF-B decoy could prevent NF-κB over-activation and partially reverse insulin resistance [33].

Vascular endothelial growth factor (VEGF)

VEGF plays a key role in diabetic vasculopathy in different organs. VEGF mediates vascular endothelial cell proliferation, migration and vasopermeability in many cells and tissues. This factor has also been found to be a primary initiator of proliferative diabetic retinopathy *in vivo* and a potential mediator of non-proliferative retinopathy. Increased serum VEGF levels

stimulate apoptosis-promoting ROS generation and cause endothelial cell activation, and thus adversely affect the endothelial cells. The balance between VEGF and angiogenic inhibitors is the chief determinant of angiogenesis and proliferation in diabetic retinopathy. Moreover, VEGF has been shown to play a role in the development of neuropathy and nephropathy in patients with diabetes. Hence, serum VEGF is a biomolecular biomarker for the severity of diabetic retinopathy [34,35].

KH902 is a VEGF receptor decoy and a fusion protein that can bind VEGF and placental growth factor (PlGF) via its binding ligand, which was taken from the VEGFR1 and VEGFR2 domains. Huang *et al.* [36] injected KH902 intravitreally into the eyes of streptozotocin-induced diabetic rats. Four weeks post-intravitreal injection, KH902-treated rats showed improvement in the retinal electrophysiological function, decreased retinal vessel leakage and reduced expression of PlGF, VEGFR2, PI3K, AKT, p-AKT, p-ERK and p-SRC compared to PBS or Avastin-treated rats. Furthermore, claudin-5 and occludin were distributed more uniformly in the retinal vessels of diabetic rats treated with KH902 rather than in those treated with Avastin or PBS [36].

A new recombinant fusion protein, named conbercept, was developed as a decoy receptor. This protein contains the second Ig domain of VEGFR1, the third and fourth Ig domains of VEGFR2, the constant region (Fc) of human IgG1, and a placental growth factor. In 2019, Zhou *et al*. [37] studied the safety and effects of this new decoy in the eyes of diabetic macular edema (DME) patients. In a retrospective clinical study, 60 eyes from patients who suffered clinically significant DME were primarily treated with at least three successive monthly intravitreal conbercept (IVC) injections. In the IVC group (n=60), the mean number of conbercept injections was 4.5±1.0 over the 12-month study. The mean central macular thickness (CMT) and best-corrected visual acuity (BCVA) increased markedly at 1 and 3 months post-IVC treatments. No serious adverse events were seen for conbercept therapy. Thus, Zhou *et al*. [37] concluded that this decoy can result in anatomic and visual improvements in DME eyes with a low number of intravitreal injections and prolonged treatment intervals in clinical practice.

TNF receptor

TNF has been shown to have a role in the pathogenesis of type 1 diabetes. TNF treatment early in life promoted diabetes in NOD mice, but when this treatment was used in older animals, TNF lowered the incidence and delayed the onset of diabetes. NOD mice with TNFR1-deficiency were completely protected from diabetes and exhibited only mild peri-insulitis. Suppression of TNF signaling seems to be beneficial in modulating the function of T regulatory cells and in inhibiting type 1 diabetes [38]. Furthermore, TNFR1 and TNFR2 (TNFR1/2) have been strongly linked with diabetes complications and heart failure: mRNA expression, protein expression and serum expression of TNFR1/2 were notably higher in patients with DM and heart failure [39].

Islet transplantation has been identified as a therapeutic approach to reduce or replace insulin in type I diabetes, but it necessitates long-term immunosuppression to circumvent

transplant rejection. Machen *et al.* [40] studied the extension of islet allograft survival by *ex vivo* gene transfer of soluble type 1 TNF receptor decoys, which resulted in islet cell cytoprotection. Following transplantation of an adenoviral vector encoding human TNFR–Ig (Ad-TNFR–Ig), the interaction of TNFα with its cell-bound receptor was prevented and the islets were protected from the effects of TNFα. This decoy method can neutralize the TNFα released by T cells and perhaps macrophages, and it elevated the threshold for beta-cell dysfunction in glucose responsiveness. The gene transfer of the Ad-TNFR–Ig decoy into human islets prevented apoptosis and cytokine-mediated beta-cell dysfunction. Furthermore, this treatment was able to improve the success of **{AuQ: Edit OK?}** allogeneic islet transplantation: diabetic mice that were transplanted with allogeneic islets expressing TNFR–Ig maintained normoglycemia markedly longer than untransduced islet recipients [40]. This work supports the practicability of using inhibitors of pro-apoptotic proteins and secreted decoys to extend islet allograft survival.

Transcription factor Sp1

Sp1, Sp3, and Sp4 are members of the Sp (specificity protein) family of transcription factors and important regulators of eukaryotic gene expression. It has been reported that Sp1 mediates the stimulation of rat calmodulin I gene expression in response to insulin [41]. Furthermore, the proangiogenic factors VEGF-A and Cyr61 contribute to neovascularization in diabetic retinopathy, and the promoters of these proangiogenic genes are responsive to Sp1. An increased amount of Sp1 binds to the promoters of these genes following high-concentration glucose treatment, and depletion of Sp1 significantly reduced their aberrant expression. Thus, further characterization of the role of Sp1 in the pathogenesis of diabetes may help to identify novel therapeutic targets [42].

Mesangial expansion, which occurs due to cell proliferation and the increased deposition of extracellular matrix proteins, is one of the first features of hyperglycemia in DM. Sp1 has been suggested to play a critical role in mesangial expansion through its transcriptional modulation of many genes involved in cell proliferation. Chae *et al.* [43] developed a phosphorothioated double-stranded Sp1-decoy oligodeoxynucleotide that could efficiently block the binding of Sp1 to the promoter zone for the transcriptional modulation of TGF-β1 and plasminogen activator inhibitor-1. This decoy oligodeoxynucleotide suppressed the transcription of these cytokines and inhibited the proliferation of primary rat mesangial cells following exposure to high glucose concentrations. Indeed, the Sp1-decoy oligodeoxynucleotide may have utility in averting the pathogenesis of renal hypertrophy [43].

In another study, Jeong *et al.* [44] investigated the effect of ring-type Sp1 decoy ODNs as important fibrogenic factors in the pathogenesis of diabetic nephropathy, looking at extracellular matrix (ECM) gene expression in streptozotocin-induced diabetic rats and cultured rat mesangial cells (RMC). The decoy ODNs were introduced into the left renal artery of diabetic rats and efficiently delivered to the kidney using hemagglutinating virus of Japan (HVJ)-liposome-mediated gene transfer. Fourteen days after injection of the R-Sp1 decoy ODN, fibronectin mRNA, type IV collagen and protein expression were significantly reduced,

and the rate of urinary creatinine excretion was decreased in the R-Sp1 decoy ODN-treated diabetic rats. The *in vivo* introduction of the R-Sp1 decoy ODN effectively decreased ECM production during nephropathy through the binding of Sp1 to the promoter zone of platelet-derived growth factor (PDGF)-induced genes. In conclusion, the R-Sp1 decoy ODN may be a powerful tool for preventing progressive diabetic nephropathy [44].

TRAIL

TRAIL or TNF-related apoptosis-inducing ligand is a member of the TNF protein superfamily. There is growing evidence that TRAIL-mediated apoptosis is not restricted to transformed cells but may also be induced in primary cells, such as immune cells. Interestingly, animal studies and a few *in vivo* studies suggest that TRAIL might protect against the development and/or progression of diabetes. As the immune system is crucially involved in diabetes development, it is likely that TRAIL may protect against diabetes through its action on innate and adaptive immunity. TRAIL is involved in the regulation of central and peripheral tolerance, mediates T cell death, inhibits cell proliferation and promotes Treg cells [45,46].

TRAIL/Apo2L is a versatile protein that modulates the homeostasis of the immune system, autoimmune diseases, infection, and apoptosis. The possible role of TRAIL in type 1 DM (T1DM) has been examined by a number of research groups. Kang *et al.* [47] showed that TRAIL had no notable cytotoxic effects on the INS-1 insulin-secreting pancreatic beta cell line. They showed that INS-1 cells were resistant to TRAIL-mediated apoptosis and exhibited changes in the expression of death and decoy TRAIL receptors **{AuQ: Edit OK?}** upon TRAIL treatment. Specifically, TRAIL treatment resulted in NF-κB translocation to the nucleus in INS-1 cells, with TRAIL-mediated NF-κB activation being preceded by IκBα degradation. Use of a pharmacological inhibitor of NF-κB, namely Bay 11-7082, blocked TRAIL-induced NF-κB translocation to the nucleus. The researchers then looked at the expression of two death receptors (DR4 and DR5) that increase TRAIL-mediated apoptosis, and two decoy receptors (DcR1 and DcR2) that inhibit TRAIL-mediated apoptosis. TRAIL treatment in INS-1 cells led to DcR1 upregulation and DR5 downregulation with no changes in the expression of DcR2 and DR4. Therefore, the resistance to apoptosis in INS-1 cells may be a result of DcR1 overexpression and DR5 underexpression, with NF-kB modulating the sensitivity of cells to TRAIL by regulating the ratio of decoy to death receptors. Thus, TRAIL may have a key role in the survival of pancreatic beta cells by controlling receptor expression in a NF-kB-dependent manner [47].

Activator protein-1 (AP-1)

Diabetic nephropathy is identified by the expansion of the glomerular mesangium, which finally results in glomerulosclerosis and may progress to renal failure. AP-1 is a transcription factor that is suggested to have a role in regulating a wide range of genes involved in cell proliferation and ECM production. Ahn *et al.* [48] studied the role of AP-1 in the expression of the ECM gene and subsequently developed a therapeutic tool based on decoy ODN. They reported that transfection with the AP-1 decoy ODN greatly inhibited angiotensin II and high-

glucose-concentration-mediated cell proliferation, as well as the expression of ECM genes in cultured mesangial cells *in vitro*. Administration of AP-1 decoy ODN into streptozotocin-induced diabetic rat kidney *in vivo* via an HVJ-liposome effectively eliminated the expression of plasminogen activator inhibitor-1 and TGF-β1. These results suggested that AP-1 activation is important for ECM production and mesangial cell proliferation in response to angiotensin II and high glucose. Furthermore, the administration of stable AP-1 decoy ODN with the highly effective HVJ liposome method represents a strong molecular therapeutic approach for preventing diabetic nephropathy [48].

MA20

The insulin Rα antibody MA-20 is a mouse monoclonal IgG2b (kappa light chain) antibody, raised against placental insulin Rα of human origin. The insulin receptor (IR) is a heterodimeric protein complex that has an intracellular beta subunit and an extracellular alpha subunit, which is disulfide-linked to a transmembrane segment. The insulin ligand binds to the IR and initiates molecular signaling pathways that promote glucose uptake in cells and glycogen synthesis. Insulin binding to IR induces the phosphorylation of intracellular tyrosine kinase domains and the recruitment of multiple SH2- and SH3-domain-containing intracellular proteins that serve as signaling intermediates for pleiotropic effects of insulin [49]

In vitro methods were used to select an RNA carrying 2'-amino pyrimidines that showed high-affinity binding to MA20, a mouse monoclonal antibody. The stability of this 2'-amino-derivatized RNA is 10,000-fold more than that of unmodified RNA in serum. This RNA can also have a role as a decoy and can prevent MA20 binding to the human insulin receptor on lymphocytes, which is its natural antigen. This RNA decoy also inhibited MA20-induced downregulation of insulin receptor expression by up to 90% in human lymphocytes in culture. Cross-reaction of this RNA decoy with autoantibodies from patients with extreme insulin resistance has been reported. Indeed, without inhibiting the binding of insulin to its receptor, the RNA decoy was able to reduce the downregulation of the insulin receptor expression by up to 80% by inhibiting anti-insulin receptor antibodies. Thus, these findings demonstrated that this *in-vitro*-isolated decoy RNA might be able to selectively and precisely block oligoclonal autoimmune responses to the self-antigens in patients with autoimmune disorders [50].

CC chemokine

The earliest reports of the importance of chemokines involved NOD mice, in which a central region of mouse chromosome 11 that is linked with diabetes was found to include what was then named the β-chemokine gene family. Most reports in type 1 diabetes, have reported chemokines that reflect those found in the NOD mouse model [49].

In 2011, Lin *et al.* [51] hypothesized that autoimmune diabetes is caused by progressive degeneration of insulin-producing beta cells in the pancreatic islets via chemokine-attracted lymphocytes. It is known that NOD mice islet cells produce chemokines during the development of autoimmune diabetes and, therefore, this research group studied the role of

inflammatory CC chemokines in the progression of diabetes in these mice. A transgenic NOD mouse was generated that overexpressed the inflammatory CC chemokine decoy receptor D6 in pancreatic islets. When compared with non-transgenic control littermates, these transgenic mice had marked reductions in the insulitis scores and frequency of diabetes. Furthermore, transgenic expression of D6 (Ccbp2) did not have any effect on systemic lymphocyte development. The transgenic expression of D6 did not change the proliferation **{AuQ: Edit OK?}** of T cell subsets such as Th1, Th2 and T regulatory cells, neither of antigen-presenting cells such as macrophages, nor of dendritic cells. The numbers and percentages and of T and B lymphocytes were diminished markedly in the pancreas. The autoantigen-specific proliferation, activation status, and diabetogenicity of lymphocytes were also significantly decreased. Lin *et al.* [51] concluded that inflammatory CC chemokines have a key role in the development of autoimmune diabetes. Transgenic expression of D6 in the pancreatic islets of NOD mice decreased this pathogenic process by halting the activation of autoreactive lymphocytes and by decreasing the migration of lymphocytes to the pancreas [51].

Conclusions

Diabetes is an important public health problem associated with considerable morbidity, mortality and a healthcare economic burden that continues to grow. There is an urgent need for novel therapeutic approaches to augment current clinical practices used to prevent diabetes-related complications and to address the increasing prevalence of this disease. Decoy-based therapy is an emerging promising strategy whose effectiveness has been shown in several diseases. In diabetes, decoy technologies, including decoy ODN and decoy peptides, could be considered as important therapeutic tools. Decoy peptides have been studied in diabetes as a way to trap Fas L, VEGF, TNF, TRAIL, and CC chemokine, whereas, NF-κB, Sp-1, AP-1 and MA20 decoys are nucleic acids that block transcription factors. Fas–FasL has been identified as particularly important because of its importance in inducing apoptosis of beta cells.

Like any new treatment, decoy-based therapy has limitations that must be overcome, including low uptake efficiency, a short half-life, and low *in vivo* stability. Therefore, improvement of the decoy technology is needed, followed by efficacy and safety evaluation in *in vitro* and *in vivo* studies. In addition, the transfection efficiency of decoys should be optimized: direct transfer of 'naked' decoy ODNs can be achieved through passive uptake, but the transfection efficiency is low and needs optimization. Cationic liposome (HVJ–liposome) or other vector systems are generally used to enhance the transfection efficiency of decoy ODNs. Moreover, because the ODN decoy takes effect in the nucleus, chaperoning of ODNs to the cell nucleus is extremely important and needs to be addressed in the future.

Conflict of interest: The authors have no conflicts of interest to declare.

References

1. Zimmet, P. *et al.* (2016) Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. *Nat. Rev. Endocrinol.* 12, 616–622
2. World Health Organization (2019) *Classification of Diabetes Mellitus*, https://www.who.int/publications/i/item/classification-of-diabetes-mellitus
3. Goyal, R. and Jialal, I. (2019) *Diabetes Mellitus Type 2*, StatPearls Publishing
4. Centers for Disease Control and Prevention (2020) *National Diabetes Statistics Report 2020,* US Department of Health and Human Services https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf
5. Njatcha, C. *et al.* (2018) STAT3 cyclic decoy demonstrates robust antitumor effects in non-small cell lung cancer. *Mol. Cancer Ther.* 17, 1917–1926
6. Nguyen, P.T. *et al.* (2018) Interaction between N-cadherin and decoy receptor-2 regulates apoptosis in head and neck cancer. *Oncotarget* 9, 31516–31530
7. Hassan, F. *et al.* (2018) Adenovirus-mediated delivery of decoy hyper binding sites targeting oncogenic HMGA1 reduces pancreatic and liver cancer cell viability. *Mol. Ther. Oncolytics* 8, 52–61
8. Cakir, I. *et al.* (2017) Soluble IL-1 decoy receptor is associated with gastric adenocarcinoma. *Turkish J. Biochem.* 42, 545–549
9. Bosch, L.J.W. *et al.* (2017) Decoy receptor 1 (DCR1) promoter hypermethylation and response to irinotecan in metastatic colorectal cancer. *Oncotarget* 8, 63140–63154
10. Kwon, Y.J. *et al.* (2017) Targeted interference of SIN3A-TGIF1 function by SID decoy treatment inhibits Wnt signaling and invasion in triple negative breast cancer cells. *Oncotarget* 8, 88421–88436
11. An, H.J. *et al.* (2015) Effects of Smad decoy ODN on shear stress-induced atherosclerotic ApoE-/-mouse. *Int. J. Clin. Exp. Pathol.* 8, 3971–3978
12. Jeong, D. *et al.* (2018) miR-25 tough decoy enhances cardiac function in heart failure. *Mol. Ther.* 26, 718–729
13. Miyake, T. *et al.* (2011) Systemic administration of ribbon-type decoy oligodeoxynucleotide against nuclear factor B and ets prevents abdominal aortic aneurysm in rat model. *Mol. Ther.* 19, 181–187
14. Chary, M.A. *et al.* (2020) COVID-19: therapeutics and their toxicities. *J. Med. Toxicol.* 16, 284–294
15. Sen, M. *et al.* (2012) First-in-human trial of a STAT3 decoy oligonucleotide in head and neck tumors: implications for cancer therapy. *Cancer Discov.* 2, 694–705
16. Mahjoubin-Tehran, M. *et al.* (2020) Decoy oligodeoxynucleotide technology: an emerging paradigm for breast cancer treatment. *Drug Discov. Today* 25, 195–200
17. Hecker, M. and Wagner, A.H. (2017) Transcription factor decoy technology: a therapeutic update. *Biochem. Pharmacol.* 144, 29–34
18. Mahjoubin-Tehran, M. *et al.* (2020) Peptide decoys: a new technology offering therapeutic opportunities for breast cancer. *Drug Discov. Today* 25, 593–598

19. Crinelli, R. *et al.* (2004) Locked nucleic acids (LNA): versatile tools for designing oligonucleotide decoys with high stability and affinity. *Curr. Drug Targets* 5, 745–752
20. Toshchakov, V.Y. and Vogel, S.N. (2007) Cell-penetrating TIR BB loop decoy peptides. *Expert Opin. Biol. Ther.* 7, 1035–1050
21. Nagata, S. and Golstein, P. (1995) The Fas death factor. *Science* 267, 1449–1456
22. Petrovsky, N. *et al.* (2002) The role of Fas ligand in beta cell destruction in autoimmune diabetes of NOD mice. *Ann. N. Y. Acad. Sci*. 958, 204–208
23. Kawamoto, K. *et al.* (2006) Adenoviral-mediated overexpression of membrane-bound human FasL and human decoy Fas protect pig islets against human CD8+ CTL-mediated cytotoxicity. *Transplant. Proc.* 38, 3286–3288
24. Huang, S.H. *et al.* (2013) Adverse effect on syngeneic islet transplantation by transgenic coexpression of decoy receptor 3 and heme oxygenase-1 in the islet of NOD mice. *Transplant. Proc.* 45, 580–584
25. Wang, Y.L. *et al.* (2010) Decoy receptor 3 protects non-obese diabetic mice from autoimmune diabetes by regulating dendritic cell maturation and function. *Mol. Immunol.* 47, 2552–2562
26. Wu, S.F. *et al.* (2004) Immunomodulatory effect of decoy receptor 3 on the differentiation and function of bone marrow-derived dendritic cells in nonobese diabetic mice: from regulatory mechanism to clinical implication. *J. Leukoc. Biol.* 75, 293–306
27. Kawamoto, K. *et al.* (2009) In vivo controlling of cellular response to pig islet xenografts by adenovirus-mediated expression of either membrane-bound human FasL or human decoy Fas. *Transplant. Proc.* 41, 331–333
28. Kawamoto, K. *et al.* (2008) Prolonged survival of pig islets xenograft by adenovirus-mediated expression of either the membrane-bound human FasL or the human decoy Fas antigen gene. *Xenotransplantation* 15, 333–343
29. Sung, H.H. *et al.* (2004) Transgenic expression of Decoy Receptor 3 protects islets from spontaneous and chemical-induced autoimmune destruction in nonobese diabetic mice. *J. Exp. Med.* 199, 1143–1151
30. Suryavanshi, S.V. and Kulkarni, Y.A. (2017) NF-κβ: a potential target in the management of vascular complications of diabetes. *Front. Pharmacol.* 8, 798
31. Indira, M. and Abhilash, P. (2013) Role of NF-Kappa B (NF-κB) in diabetes. *Onco Therapeutics* 4, 111–132
32. Quan, N. *et al.* (2001) Administration of NF-kappaB decoy inhibits pancreatic activation of NF-kappaB and prevents diabetogenesis by alloxan in mice. *FASEB J.* 15, 1616–1618
33. Zhong, L. *et al.* (2011) Effect of NF-κB decoy on insulin resistance of adipocytes from patients with type 2 diabetes mellitus. *Diabetes Metab.* 37, 520–526
34. Aiello, L.P. and Wong, J.-S. (2000) Role of vascular endothelial growth factor in diabetic vascular complications. *Kidney Int.* 58, S113–S119

35. Ahuja, S. *et al.* (2019) Serum vascular endothelial growth factor is a biomolecular biomarker of severity of diabetic retinopathy. *Int. J. Retina Vitreous* 5, 29
36. Huang, J. *et al.* (2012) Effects of intravitreal injection of KH902, a vascular endothelial growth factor receptor decoy, on the retinas of streptozotocin-induced diabetic rats. *Diabetes Obes. Metab.* 14, 644–653
37. Zhou, Q. *et al.* (2019) One-year outcomes of novel VEGF decoy receptor therapy with intravitreal conbercept in diabetic retinopathy-induced macular edema. *Mol. Vis.* 25, 636–644
38. Chee, J. *et al.* (2011) TNF receptor 1 deficiency increases regulatory T cell function in nonobese diabetic mice. *J. Immunol.* 187, 1702–1712
39. Ping, Z. *et al.* (2017) TNF receptor 1/2 predict heart failure risk in type 2 diabetes mellitus patients. *Int. Heart J.* 58, 245–249
40. Machen, J. *et al.* (2004) Prolongation of islet allograft survival following ex vivo transduction with adenovirus encoding a soluble type 1 TNF receptor-Ig fusion decoy. *Gene Ther.* 11, 1506–1514
41. Pan, X. *et al.* (2001) Insulin deprivation leads to deficiency of Sp1 transcription factor in H-411E hepatoma cells and in streptozotocin-induced diabetic ketoacidosis in the rat. *Endocrinology* 142, 1635–1642
42. Donovan, K. *et al.* (2013) Role of Sp1 transcription factor in the pathogenesis of diabetic retinopathy. *Invest. Ophthalmol. Vis. Sci.* 54, 5605
43. Chae, Y.M. *et al.* (2004) Sp1-decoy oligodeoxynucleotide inhibits high glucose-induced mesangial cell proliferation. *Biochem. Biophys. Res. Commun.* 319, 550–555
44. Jeong, H.K. *et al.* (2008) Suppression of mesangial cell proliferation and extracellular matrix production in streptozotocin-induced diabetic rats by Sp1 decoy oligodeoxynucleotide in vitro and in vivo. *J. Cell. Biochem.* 103, 663–674
45. Bossi, F. *et al.* (2015) TRAIL modulates the immune system and protects against the development of diabetes. *J. Immunol. Res.* 2015, 680749
46. Lorz, C. *et al.* (2008) The death ligand TRAIL in diabetic nephropathy. *J. Am. Soc. Nephrol.* 19, 904–914
47. Kang, S. *et al.* (2010) TRAIL upregulates decoy receptor 1 and mediates resistance to apoptosis in insulin-secreting INS-1 cells. *Biochem. Biophys. Res. Commun.* 396, 731–735
48. Ahn, J. *et al.* (2004) Transcription factor decoy for AP-1 reduces mesangial cell proliferation and extracellular matrix production in vitro and in vivo. *Gene Ther.* 11, 916–923
49. Forsayeth, J.R. *et al.* (1987) Effect of monoclonal antibodies on human insulin receptor autophosphorylation, negative cooperativity, and down-regulation. *J. Biol. Chem.* 262, 4134–4140
50. Lee, S.W. and Sullenger, B.A. (1996) Isolation of a nuclease-resistant decoy RNA that selectively blocks autoantibody binding to insulin receptors on human lymphocytes. *J. Exp. Med.* 184, 315–324

51. Lin, G.J. *et al.* (2011) Transgenic expression of murine chemokine decoy receptor D6 by islets reveals the role of inflammatory CC chemokines in the development of autoimmune diabetes in NOD mice. *Diabetologia* 54, 1777–1787

Table 1. Summary of decoy-based research for diabetes treatment.

Decoy name	Up/ downregulation of the target	Concentration /dose and time µM/mg/kg/day	Decoy type	Model/cell line	Delivery method	Diabetes type	Results	Reference
Human decoy Fas antigen	↓ Fas–FasL	Multiplicity of infection (MOI) of 100 for 1 hour	Peptide	Pig islet cells	Adenovirus vectors	Type 1 diabetes mellitus	Protection of pig islets from human CD8 cytotoxic T lymphocyte (CTL)-mediate cytotoxicity and improve xenograft survival	[23]
Decoy Receptor 3	↓ Fas ligand and LIGHT	–	Peptide	Islet of non-obese diabetic (NOD) mice	Microinjected into the pronuclei of NOD embryos at the one-cell stage, then implanted into pseudo pregnant F_1 females	Autoimmune diabetes	Double-transgenic islets that co-express HO-1 and DcR3 did not result in a better outcome	[24]
Decoy Receptor 3	↓ Fas ligand and LIGHT	100 µg	Peptide	NOD/Sytwu (Kd, Db, Ld, I-Ag7) and NOD/SCID mice	Lipofectamine	Autoimmune diabetes	Protects NOD mice from autoimmune diabetes	[25]
Decoy receptor 3	↓ Fas ligand and LIGHT	–	Peptide	Bone marrow-derived dendritic cells in NOD mice	Intravenous injection	Autoimmune diabetes	Confirmed the action of cytokine-modulated dendritic cells (DCs) in the induction and maintenance of tolerance	[26]
Membrane-bound	↓ Fas–FasL	–	Peptide	Pig islet xenografts	Adenoviral transfer	Type 1 diabetes	Control cellular response to pig	[27]

human FasL or human decoy Fas							islet xenografts. Prolongs pig islet xenograft survival	
Membrane-bound human FasL or the human decoy Fas antigen gene	↓ Fas–FasL	–	Peptide	Preimmunized diabetic rats	Adenoviral transfer	Type 1 diabetes	Prolonged survival of pig islets xenograft Prevention of CTL-mediated xenocytotoxicity in pig islets	[28]
DCR3	↓ Fas ligand and LIGHT	–	Receptor	Transgenic NOD mice	Embryo microinjection	Autoimmune diabetes	Immune-evasion function of DCR3 inhibits autoimmunity, and genetic manipulation of grafts may improve the success and survival of islet transplants	[29]
NF-κB decoy	↓ NF-κB	10 μg	Oligodeoxynucleotide (ODN)	CD-1 mice	Intravenous injection	Insulin-dependent diabetes mellitus	Inhibit pancreatic activation of NF-κB and prevents diabetogenesis by alloxan	[32]
NF-kB decoy	↓ NF-κB	4.0 μg	ODN	Subcutaneous abdominal adipose tissue	Lipofectamine 2000	Type 2 diabetes mellitus	Inhibition of NF-kB overactivation and also partly reversed insulin resistance	[33]
KH902	↓ VEGF	20 mg/ml	Peptide	Retinas of streptozotocin-induced diabetic rats	Intravitreal injection	–	Improved retinal electrophysiological function and inhibited the breakdown of inner blood-retina barrier (iBRB)	[36]
Vascular endothelial-derived growth factor (	↓ VEGF	0.5 mg in 0.05 ml	Peptide	Eyes of patients with diabetic macular	Intravitreal injection	Type 1 or type 2 diabetes mellitus	The decoy is effective and safe for the treatment of a	[37]

VEGF) decoy				edema (DME)			majority of patients with DME	
Tumor necrosis factor receptor (TNFR)–Ig	↓ Tumor necrosis factor (TNF)	Plaque forming unit (PFU of $1x10^4$ to $1x10^6$	Peptide	Human islets	Adenoviral transfer	Type I diabetes	Facilitating allogeneic islet transplantation	[40]
Sp1-decoy	↓ Transcription factor Sp-1	100 nM	Phosphorothioated ODN	Rat mesangial cells	LipofectAMINE Plus	Diabetes mellitus	Inhibiting high glucose-induced mesangial cell proliferation and preventing the pathogenesis of renal hypertrophy	[43]
Sp1-decoy	↓ Transcription factor Sp-1	0.5 mg	ODN	Streptozotocin-induced diabetic rats	Hemagglutinating virus of Japan (HVJ)-liposome mediated gene transfer and LipofectAMINE Plus	Diabetic nephropathy	Suppression of mesangial cell proliferation and extracellular matrix production	[44]
Decoy receptor 1	↓ TRAIL	–	Peptide	Insulin-secreting pancreatic beta cell line, INS-1	–	Type 1 diabetes	Act as dominant-negative inhibitors of TRAIL-mediated apoptosis	[47]
AP-1	↓ Activator protein-1	–	ODN	Cultured human vascular smooth muscle cells	HVJ - liposomes	Diabetes mellitus	High glucose and Ang II stimulated PAI-1 expression via AP-1 biding sites.	[48]
Nuclease-resistant MA20 decoy	↑ MA20	30 µg	2'-amino-derivatized RNA	IM-9 human lymphoblasts	–	Autoimmune diabetes	Block MA20 binding to its natural antigen, the human insulin receptor, on lymphocytes	[50]
DCRD6	↓ CC chemokine	–	Peptide	NOD mice	D6 DNA was purified and microinjected into the pronuclei of	Autoimmune diabetes	The frequency of diabetes and insulitis scores of transgenic mice were	[51]

					NOD embryos at the one-cell stage		decreased significantly	

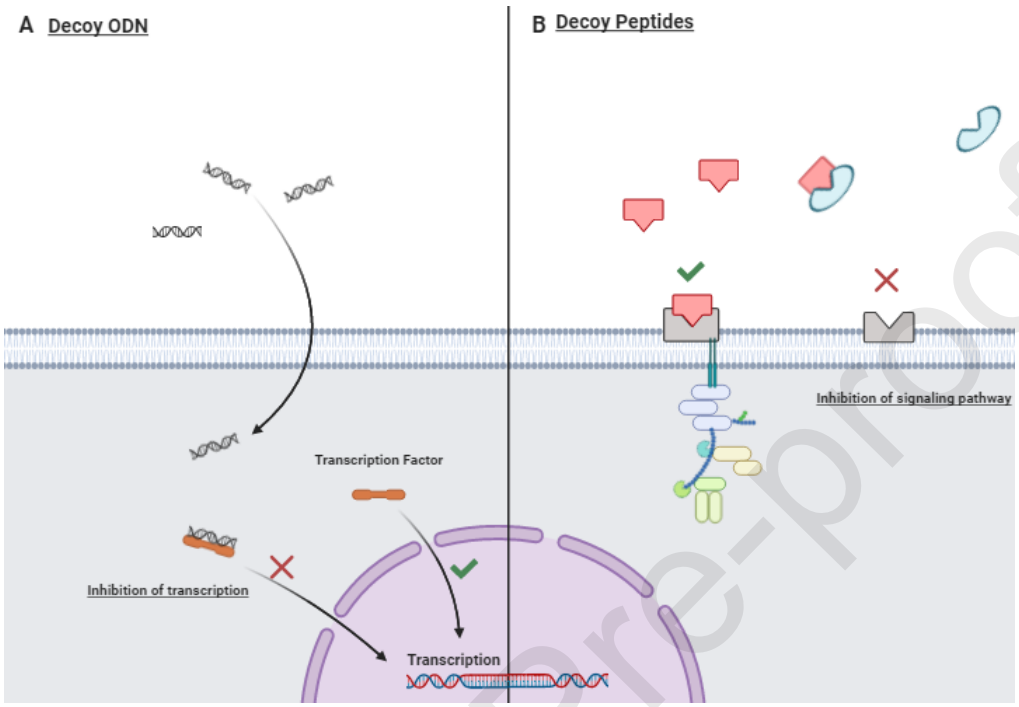


Figure 1: Schematic illustration of the mechanisms of decoy technology. **(a)** A decoy can bind to a specific transcription factor and inhibit the transcription of the target gene. **(b)** Decoy peptides act as a molecular trap that can bind to specific ligands and reduce normal signaling.

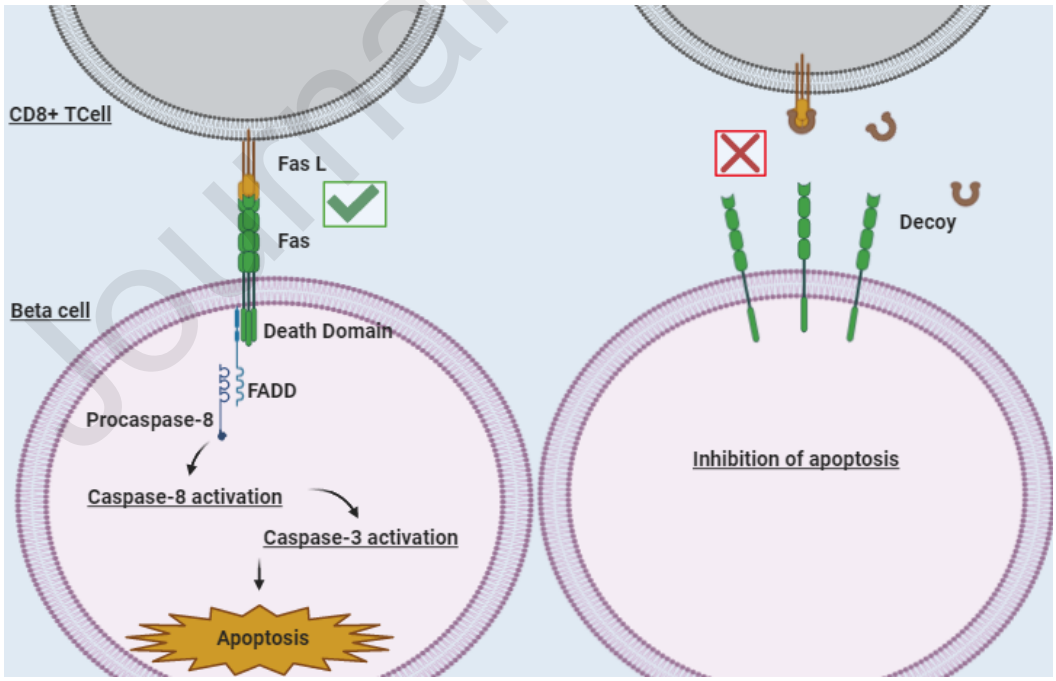


Figure 2: Application of decoy peptides for inhibition of the Fas–FasL pathway as a promising approach in the treatment of type 1 diabetes mellitus (T1DM). Beta cell destruction by islet-

reactive T cells, a process related to beta cell apoptosis in T1DM. Decoy peptides act by blocking FasL on the T cells to reduce the apoptosis of beta cells.

Highlights

- There is an urgent need for novel therapeutic approaches against diabetes.
- Decoy-based therapy is an emerging promising strategy for diabetes and its complications.
- Decoy technology comprises the use of decoy oligodeoxynucleotides and decoy peptides.
- This review summarizes the therapeutic effects of decoy-based therapies in diabetes.