A Systematic Review of the Efficacy of Gene Therapy for Diabetes and Diabetes-Related Complications

By

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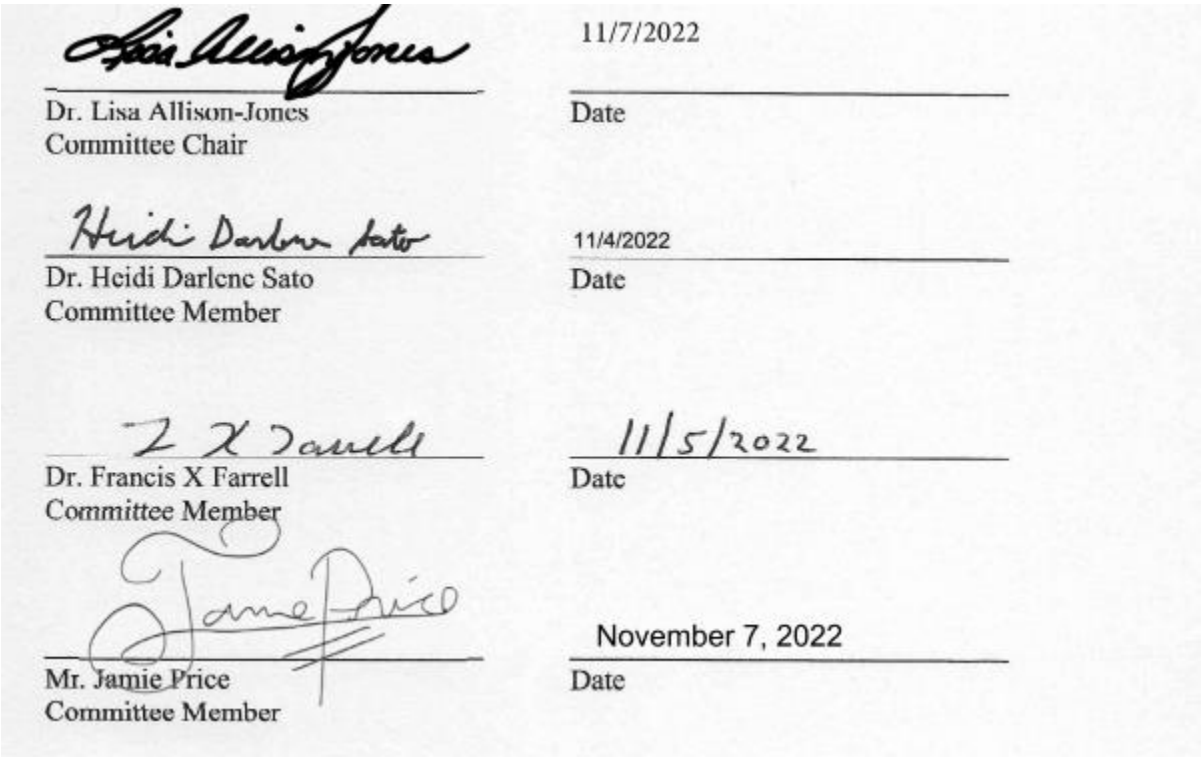

Abstract

Objectives: Diabetes is a chronic condition characterized by excess blood sugar in genetically predisposed individuals. It is one of the top 10 causes of mortality and the top six causes of morbidity. The number of diabetes cases has tripled over the last 20 years and is projected to increase another 51% by 2045. It is evident that current methods of diabetes treatment have failed to stop this global crisis. Researchers are now pursuing gene therapy to find more effective treatments for diabetes. Numerous systematic reviews addressed the efficacy of pharmaceutical agents for treating diabetes in humans. However, no systematic review on the effectiveness of gene therapy for diabetes in humans was found. The purpose of this project was to conduct a systematic review to address this gap in the literature and determine whether gene therapy is an effective diabetes treatment.

Methodology*:* The conventional steps of a systematic review were followed for this project. First, the PICO format was used to formulate focused review questions and define the inclusion and exclusion criteria. Then, an iterative search strategy was utilized to find peer-reviewed journal articles and grey literature from ten 10 preselected databases. Articles were selected based on predetermined inclusion and exclusion criteria. The selection process was reported using a PRISMA flow chart. A kappa calculation was conducted to determine the inter-rater reliability of the selection process, and the quality of the selected studies was assessed using a Jadad scale. The selected studies were divided into sections by type of diabetes and related complications, then subsections based on the gene therapy pathways utilized in the studies and synthesized.

Results: Forty-seven ongoing and completed trials were deemed suitable for inclusion in the systematic review. The kappa coefficient for the selection process was 0.93, indicating that interrater agreement was almost perfect. The median Jadad score was 4 with an interquartile

range of 4, suggesting that the included studies were of high quality and had a low risk of bias. Fourteen out of the 16 completed trials with gene therapy for type 1 diabetes, four out of the five completed trials for type 2 diabetes, and 12 out of 15 completed studies on diabetes-related complications yielded positive results.

Conclusions: This project addressed the gap in the literature by conducting a systematic review of 47 clinical trials on gene therapy for diabetes in humans. For type 1 diabetes, gene therapy trials to induce self-tolerance via T regulatory cells, interleukin cells, dendritic cells, genetically modified proinsulin, and monoclonal antibodies were particularly effective. For type 2 diabetes, gene therapy with glucokinase activators to increase insulin production and mesenchymal cell therapy to repair damaged beta cells demonstrated the most success. Gene therapy with growth factors successfully induced angiogenesis and treated diabetic neuropathy and critical limb ischemia. Gene therapy to inhibit vascular endothelial growth factors worked to treat diabetic vascular eye disorders. Overall, the gene therapy studies in this systematic review provided evidence that gene therapy is an effective treatment for treating type 1 and 2 diabetes and diabetes-related complications.

Keywords: *systematic review, gene therapy, diabetes, human, clinical trial*

Acknowledgments

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Dedication

To all the scientists who dared to dream of a cure for diabetes and who are now working tirelessly to realize that dream.

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Chapter One

Introduction

Diabetes mellitus (DM), commonly known as “diabetes,” refers to a group of diseases that affect how the body turns food into energy. Diabetes is one of the top 10 causes of mortality worldwide. According to the International Diabetes Federation (2019a), 4.2 million deaths were caused by diabetes in 2019 alone. This number equates to one death per eight seconds. Almost half of these deaths occurred in people under 60 years of age (International Diabetes Federation, 2019a; Williams et al., 2020). Overall, people with diabetes face a 15% increased risk of all-cause mortality compared to people who do not have diabetes (Chatterjee et al., 2017); specifically, this risk doubles in people who are younger than 55 years (International Diabetes Federation, 2019b; Williams et al., 2020). Globally, diabetes is one of the top six causes of disability (International Diabetes Federation, 2020). Diabetes causes both acute and chronic illnesses, which can lead to permanent illness or even death (International Diabetes Federation, 2019b; Lin et al., 2020). Long-term complications of diabetes include nerve disease, vision loss, cardiovascular diseases, kidney failures, and gum diseases (International Diabetes Federation, 2019a). One in every 11 people in the world lives with diabetes, and the impact on health and finance is devastating (International Diabetes Federation, 2020). The search for a new treatment is ongoing; gene therapy is one approach. The purpose of this project is to conduct a systematic review to determine whether gene therapy is an effective diabetes treatment. In this chapter, the classification, diagnosis, and treatment of diabetes will be discussed, the prevalence of diabetes and its economic impact will be examined, and the purpose of this systematic review will be considered.

Classification and Diagnosis of Diabetes

The most prevalent forms of diabetes are type 1, type 2, and gestational diabetes (American Diabetes Association, 2019a). Complete deficiency is classified as type 1, progressive insulin deficiency is classified as type 2, and hyperglycemia during the second or third semester of pregnancy is classified as gestational diabetes (International Diabetes Federation, 2019a). Additionally, some other forms of diabetes exist in only 1.5-2% of the world's population. These are latent autoimmune diabetes in adults, maturity-onset diabetes of the young, maternally inherited diabetes and deafness, and neonatal diabetes and diabetes that occur due to other diseases such as pancreatitis and cystic fibrosis (Shoily et al., 2021).

Regardless of the type of diabetes, diagnosis of diabetes or prediabetes is based on a person's blood sugar level. High blood sugar (hyperglycemia) is determined through hemoglobin A1C, random plasma glucose levels, fasting plasma glucose (FPG), or the two-hour plasma glucose value after a 75-gram oral glucose tolerance test (OGTT). Table 1.1 provides the criteria used to diagnose diabetes and prediabetes by the American Diabetes Association (ADA), European Association for the Study of Diabetes, and the American Association of Clinical Endocrinologists (AACE) (American Diabetes Association, 2019a; International Diabetes Federation, 2019a).

Table 1.1

Diabetes Classification

	Normal	Prediabetes	Diabetes
A1C	≤5.6 %	5.7-6.4 %	≥6.5 %
FPG	≤99 mg/dL	100-125 mg/dL (5.6-6.9 mmol/L)	≥ 126 mg/dL (7.0 mmol/L)
OGTT	1313≤139 mg/dL	100-140-199 mg/dL (7.8-11.0 mmol/L)	≥ 200 mg/dL (11.1 mmol/L)*
RPG	RPG		≥ 200 mg/dL (11.1 mmol/L)**

*In the absence of unequivocal hyperglycemia, results should be confirmed by repeat testing.
**Only diagnostic in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis.
RPG, random plasma glucose.

Note: Diagnosis of prediabetes and diabetes. Adapted from "Classification and Diagnosis of Diabetes" from the International Diabetes Federation (https://diabetesatlas.org/upload/resources/previous/files/8/IDF_DA_8e-EN-final.pdf). Copyright 2021 by the International Diabetes Federation.

As listed in Table 1.1, diabetes is diagnosed based on one or more of these tests: Fasting plasma glucose is 7.0 mmol/L or higher, hemoglobin A1C is higher than 6.5%, 2-hour plasma glucose after ingesting 75 g of a glucose solution at 11.1 or higher and random plasma glucose 11.1 mmol/L. Prediabetes is diagnosed based on one or more of the criteria fasting plasma glucose between 5.7-6.9 mmol/L and 2-hour plasma glucose after ingestion of 75 g glucose solution at 7.8 to 11.0 mmol/l and hemoglobin A1C level between 5.7 to 6.4% (American Diabetes Association, 2019a; International Diabetes Federation, 2019a).

Current Diabetes Treatments

When considering conventional treatments, patients with diabetes are divided into two main categories: insulin-dependent and non-insulin-dependent. Insulin-dependent diabetes primarily includes patients with type 1 diabetes, although some patients with type 2 diabetes, gestational diabetes, and other types of diabetes also require daily insulin treatments. Currently,

insulin treatments come in different types: rapid-acting, short-acting, intermediate-acting, and long-acting, and various modes such as injections, pumps, and inhalers. Additionally, whole pancreas or islets are being transplanted in insulin-dependent patients with diabetes to replace dysfunctioning beta cells with functioning beta cells to increase insulin production (National Institute of Diabetes and Digestive and Kidney Diseases, 2016).

Non-insulin-dependent patients are treated with therapies other than insulin as insulin, such as sensitizers, secretagogues, alpha-glucosidase inhibitors, biguanides, incretins, pramlintide, bromocriptine, and sodium-glucose cotransporter 2 (SGLT-2) inhibitors. These medications work in different mechanisms to lower blood sugar by increasing insulin sensitivity of peripheral tissues, increasing insulin secretion by beta cells, reducing hepatic glucose production, or reducing carbohydrate absorption by the intestines (National Institute of Diabetes and Digestive and Kidney Diseases, 2016).

One potential method of treating diabetes is gene therapy, a technique that repairs or reconstructs defective genetic material to treat or prevent disease. Gene therapy with glucagon-like peptide-1 (GLP-1) has shown success in treating type 2 diabetes by increasing gene expression, stimulating insulin secretion, and aiding in beta-cell survival (National Institute of Diabetes and Digestive and Kidney Diseases, 2016; U.S Food and Drug Administration, 2019a).

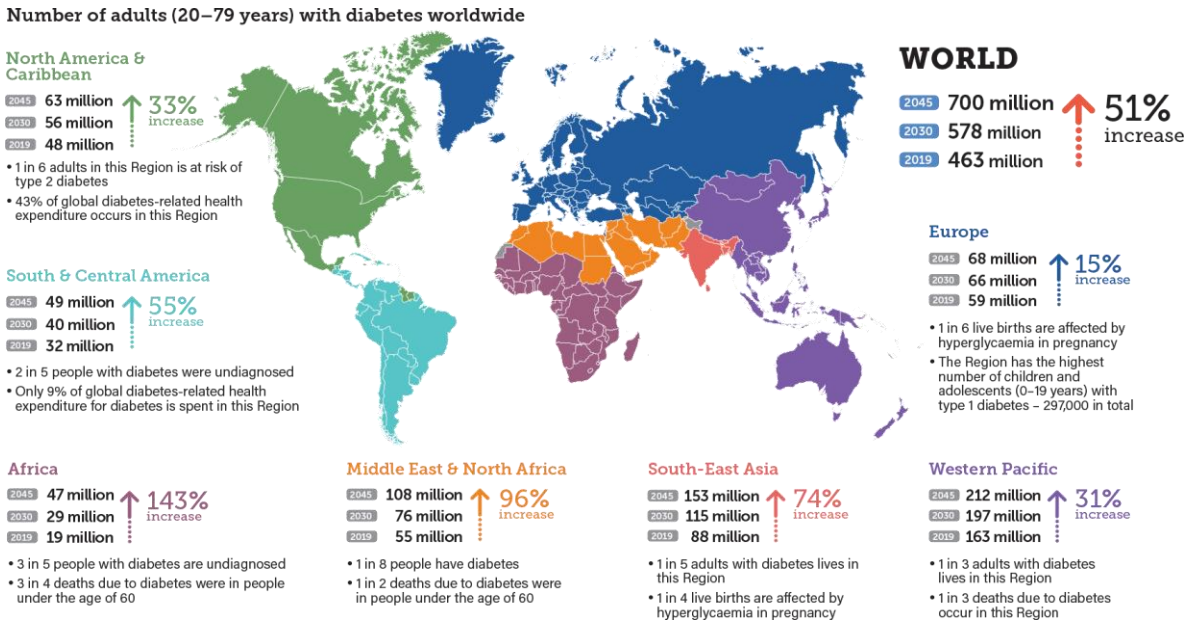
Other approaches to gene therapy, such as reprogramming human alpha cells to beta cells to produce insulin (Osipovich & Magnuson, 2018), modification of porcine and rodent beta cells to produce human insulin (Bellin & Dunn, 2020), and cellular regeneration of beta cells through proliferation, neogenesis, and transdifferentiation (Guney et al., 2020; Qadir et al., 2020) are also under investigation.

Prevalence of Diabetes

Diabetes is considered one of the fastest-growing health problems of this century, as the number of cases has more than tripled over the last 20 years (International Diabetes Federation, 2019b; Lin et al., 2020). The International Diabetes Federation predicted in 2010 that global diabetes prevalence will be 438 million by 2025. Unfortunately, global diabetes prevalence surpassed this number by 25 million in 2019, six years before the predicted time. If this trend continues, the International Diabetes Federation estimates that there will be 578 million adults with diabetes by 2030 and 700 million by 2045 (International Diabetes Federation, 2020; Lin et al., 2020; Williams et al., 2020).

Figure 1.1

The Global Rate of Increase in Diabetes



Note: Shows projected increase in diabetes cases all over the world with an overall 51% increase. Adapted from "Global Diabetes Data Report 2010-2045" by International Diabetes Federation (https://diabetesatlas.org/data/en/world/). Copyright 2020 by the International Diabetes Federation.

As illustrated in Figure 1.1, the number of diabetes cases in people aged 20-79 is projected to increase in all parts of the world with a 33% increase in North America and the Caribbean, 55% increase in South and Central America, 143% in Africa, 96% in the Middle East and North Africa, 74% in Asia, 15% in Europe, and 31% in the Western Pacific, with a 51% overall increase by 2045 (International Diabetes Federation, 2020; Lin et al., 2020; Williams et al., 2020).

Economic Impact of Diabetes

Diabetes complications and treatments come with a tremendous economic impact. People with type 1 diabetes produce almost no insulin and need daily insulin injections to survive. In addition, they need to test their blood sugar regularly to maintain safe blood glucose levels. In most countries, access to insulin injections and testing supplies is limited and expensive. Unfortunately, without appropriate blood sugar management, harmful substances known as "ketones" can build up and lead to diabetic ketoacidosis (DKA), which may cause severe disability and even early death. In addition to DKA, people with type 1 diabetes risk abnormally low blood glucose (hypoglycemia) and early onset of vascular complications. Children with type 1 diabetes have poor metabolic control and poor growth. Living with this condition is challenging for the child and the whole family. With type 2 diabetes, lifestyle changes and oral medication such as metformin, a drug that works by reducing hepatic glucose production, are prescribed initially (American Diabetes Association, 2022b). In later stages of type 2 diabetes, insulin injections might be necessary, which can be costly in most countries (Williams et al., 2020; Zheng et al., 2018).

People with diabetes incur high healthcare costs as they are two to three times more likely to suffer from cardiovascular disease (CVD) and 10 times more likely to suffer from end-

stage renal disease (ESRD) (Williams et al., 2020). CVD-related care represents the most significant proportion of diabetes health expenditures. One out of four diabetes inpatient costs are a consequence of CVD, and 15% of the expenses of physician office visits are related to CVD (Williams et al., 2020; Zheng et al., 2018).

Chronic kidney disease (CKD) is another diabetes-related complication associated with significant additional health expenditure. People with CKD tend to require dialysis and kidney transplants and incur high costs associated with this process. Initial stages of dialysis may cost $40,000/year, and end-stage renal disease dialysis may cost $100,000/year. In addition, people with CKD or caregivers of a child with CKD find it difficult to maintain full-time employment and experience high opportunity costs due to job losses (Silva et al., 2018). Overall, people with diabetes and CKD experience 50% higher health expenditures compared to those with diabetes but without CKD. Most people lack access to dialysis and kidney transplants (International Diabetes Federation, 2018; Williams et al., 2020).

Feet and vision problems are some of the most severe complications of diabetes and a leading cause of disability (Lazzarini et al., 2018). People with foot ulcers experience five times higher health expenditures than those without foot ulcers (Lazzarini et al., 2018; P. Zhang et al., 2017). One in three people with diabetes experience partial or complete vision loss and incur costs due to absence from the labor force, treatment, and requiring support from caregivers (International Diabetes Federation, 2019b).

On average, people with diagnosed diabetes have medical expenditures approximately twofold higher than people without diabetes (Lin et al., 2020; World Health Organization, 2018b). Consequently, diabetes negatively impacts a patients and a country’s economic wellbeing. The top 10 countries with the highest national diabetes-related health expenditure

were the United States of America, China, Brazil, Germany, Japan, Mexico, France, United Kingdom, Canada, and Russia, respectively (Williams et al., 2020), as illustrated in Table 1.2.

Table 1.2

Countries With the Highest Diabetes-Related Health Expenditures

Top 10 countries or territories for total health expenditure (billion USD) due to diabetes (20–79 years) in 2019 and top 10 countries or territories for mean annual diabetes-related health expenditure per person.				
Rank	Country or territory	Total national diabetes-related health expenditure in 2019 (billion USD), people aged 20–79	Country or territory	Mean annual health expenditure per person with diabetes (USD), people aged 20–79
1	United States of America	294.6	Switzerland	11,915.6
2	China	109.0	United States of America	9505.6
3	Brazil	52.3	Norway	9061.4
4	Germany	43.8	Luxembourg	7977.8
5	Japan	23.5	Sweden	6643.1
6	Mexico	17.0	Ireland	6597.6
7	France	16.9	Iceland	6403.1
8	United Kingdom	14.1	Denmark	5521.1
9	Canada	12.3	Netherlands	5379.7
10	Russian Federation	10.6	Austria	5259.3

Note: Data compiled from “Top 10 countries or territories for total health expenditure (billion USD) due to diabetes (20–79 years) in 2019” and “Top 10 countries or territories for mean annual diabetes-related health expenditure per person” by the Diabetes Research and Clinical Practice. Copyright 2019 by the Diabetes Diabetes Research and Clinical Practice.

Overall, diabetes and its complications profoundly impact the global economy. In 2019, the global financial cost of diabetes was $760 billion. There are direct and indirect costs associated with diabetes. The direct cost refers to diabetes-related complications such as diabetes ketoacidosis (DKA). The average cost of treatment for each DKA episode is $1750 in the United Kingdom and $29,981 in the United States of America (International Diabetes Federation, 2019a; Lyerla et al., 2021). On the other hand, indirect costs are associated with diabetes, including labor-force dropout, mortality, and reduced productivity at work. In 2017, the estimated global direct healthcare costs from diabetes reached $727 billion, and direct and indirect cost combined was $1.3 trillion (Bommer et al., 2017; International Diabetes Federation, 2019a).

Statement of the Problem

Diabetes is one of the top 10 causes of death worldwide and a significant contributor of morbidity. Generally, people with diabetes face a 15% increased risk of all-cause mortality than people who do not have diabetes (Chatterjee et al., 2017). As world population continues to grow and age, the prevalence of diabetes and its economic impact are projected to increase at an alarming rate. The International Diabetes Federation projects that the number of adults with diabetes to increase to 578 million by 2030 and 700 million by 2045 and the cost of care to increase to $825 billion by 2030 and $845 billion by 2045 (International Diabetes Federation, 2019b, 2020; Lin et al., 2020; Williams et al., 2020).

The significant personal and global economic impact of diabetes makes it important to research for an effective treatment (International Diabetes Federation, 2019b). Current therapeutic approaches increase insulin output, improve insulin sensitivity through medications during the initial stages of diabetes, and replace insulin during later stages of diabetes (National

Institute of Diabetes and Digestive and Kidney Diseases, 2016; White, 2014). While these approaches allow disease maintenance, they do not cure diabetes. Over time, people with diabetes face long-term microvascular complications such as retinopathy, nephropathy, and neuropathy and macrovascular complications such as cardiovascular and cerebrovascular diseases. These complications increase mortality, kidney failure, blindness, and decreased quality of life (American Diabetes Association, 2019b; International Diabetes Federation, 2020). All diabetes is associated with a decline in insulin production (National Institute of Diabetes and Digestive and Kidney Diseases, 2016). Therefore, gene therapy targeted to intensify insulin production has the potential to cure this disease.

Purpose of Review and Review Questions

The purpose of this systematic review is to determine whether gene therapy is an effective diabetes treatment. Following are the review questions will be considered for the project.

RQ1: In patients with type 1 diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes (O)?

RQ2: In patients with type 2 diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes (O)?

RQ3: In patients with diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes-related neural/nerve disorders (O)?

RQ4: In patients with diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing critical limb ischemia (O)?

RQ5: In patients with diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes-related eye complications (O)?

In this chapter, an overview of diabetes, its impact on morbidity and mortality, and the economic costs of managing the disease were discussed, and the review questions guiding the study were presented. The next chapter will provide a literature review around this topic.

Chapter Two

Review of the Literature

The treatment goals of diabetes involve eliminating the symptoms related to hyperglycemia and reducing long-term complications. Treatment options for diabetes have evolved over the past century and are still growing. This chapter will first provide an overview of pathophysiology and genetics of different types of diabetes, and various diabetes medications. Then gene therapy methods and current gene therapy treatments will be described. Finally, the possibility of gene therapy for diabetes will be explored.

Pathophysiology and Genetics of Different Types of Diabetes

Gene therapy for diabetes and its complications has attracted intensive interest in recent years. For appropriate treatment for any disease, it is essential to know the pathophysiology of a disease. Additionally, it is crucial to know which genes are responsible for the illness for appropriate gene therapy to occur. Therefore, this section will discuss the pathophysiology and genetics of different types of diabetes.

Type 1 Diabetes

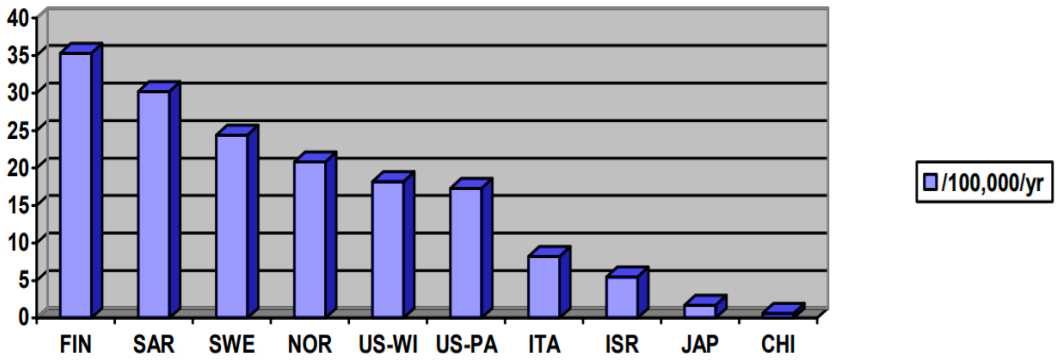
Type 1 diabetes is caused by the autoimmune destruction of the insulin-producing beta cells in the pancreas. Infective agents trigger this autoimmune destruction in genetically predisposed individuals. Without functioning beta cells, insulin cannot be produced. Therefore, people with type 1 diabetes entirely depend on exogenous (outside) insulin resources. Type 1 is the most common form of diabetes in children and adolescents. Genetic predisposition is an essential factor in developing type 1 diabetes. It is a heritable polygenic disease with identical twin concordance of 30-70%, siblings 6-7% and 1-9% with one parent with type 1 diabetes (DiMeglio et al., 2018; Redondo et al., 2018). So far, over 73 genes have been suspected to be involved in developing type 1 diabetes. These genes include human leukocyte antigens, insulin

genes, and cytotoxic T lymphocyte-associated antigen-4 genes. Mutations in these genes likely produce an inadequate immune response in the pancreas that destroys beta cells and induces insulin deficiency and hyperglycemia (Yahaya & Salisu, 2021). Two human leukocyte antigens (HLA) on chromosome 6 contribute to approximately 50% of the familial cases of type 1 diabetes and are most prevalent in the Caucasian population. About 90% of the children with type 1 diabetes have HLA genes DR4-DR 8 and DR3-DQ2 (DiMeglio et al., 2018; Redondo et al., 2018).

Type 1 diabetes accounts for about 10% of all diabetes cases. The prevalence of type 1 diabetes is slightly more common in men and boys than women and girls. Additionally, it is more prevalent in countries with cold weather, has higher incidences during winter than summer, and is more common in the Caucasian population. As illustrated in Figure 2.1, the countries with long winters and Caucasian populations have the highest prevalence of type 1 diabetes (World Health Organization, 2018a). Because of this, researchers speculate that cold weather may trigger this disease (DiMeglio et al., 2018; Redondo et al., 2018). Moreover, type 1 diabetes is more prevalent in children fed cow's milk or formula and solid foods than breastfed children. Therefore, there is speculation that this type of feeding may trigger autoimmune disorders and the destruction of beta cells in children (American Diabetes Association, 2021b; Redondo et al., 2018).

Figure 2.1

Prevalence of Type 1 Diabetes



FIN = Finland, SAR = Sardinia, SWE = Sweden, NOR = Norway, US-WI = US-Wisconsin, US-PA = US-Pennsylvania, ITA = Italy, ISR = Israel, JAP = Japan, CHI = China

Note: Shows higher type 1 diabetes prevalence in cold climates. Adapted from "Genetics and Diabetes" by World Health Organization, 2021 (https://www.who.int/genomics/about/Diabetis-fin.pdf). Copyright 2021 by the World Health Organization.

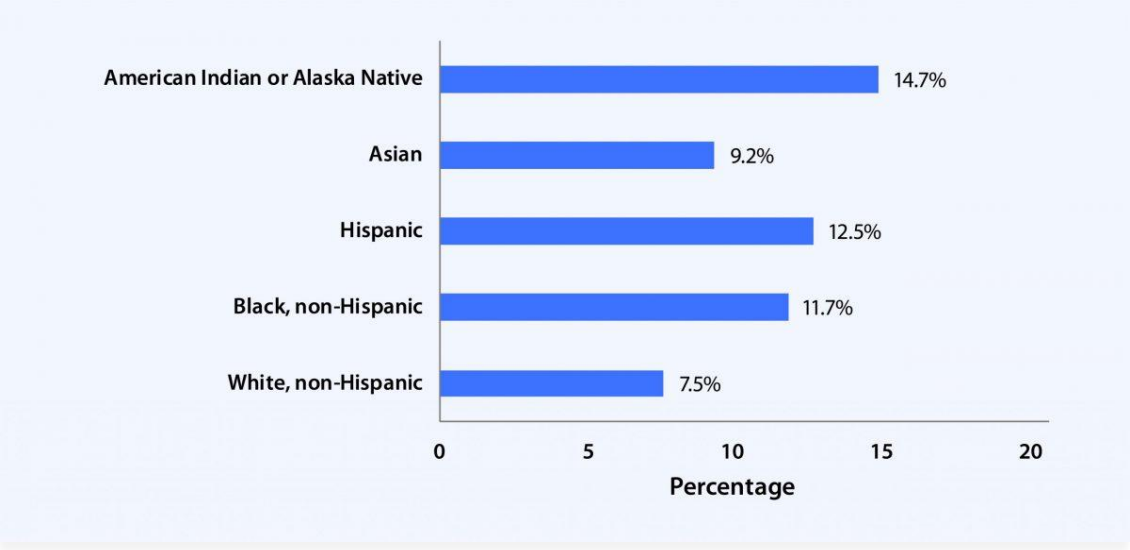
Type 2 Diabetes

Type 2 diabetes accounts for about 90% of diabetes cases worldwide. It is considered a metabolic disorder caused by dysfunctional beta cells to produce an appropriate amount of insulin and the inability of insulin-sensitive tissues to respond to insulin. Defects in both mechanisms cause a metabolic imbalance leading to high blood sugar levels and pathogenesis of type 2 diabetes (American Diabetes Association, 2021b). Type 2 diabetes is more common in African Americans, Hispanics/Latinos, American Indians, Asian Americans, and Pacific Islanders than non-Hispanic Whites. As illustrated in Figure 2.2, American Indian or Alaska Native adults have the highest rates of diagnosed type 2 diabetes (14.7%) among all U.S. racial and ethnic groups, followed by Hispanics (12.5%) and non-Hispanic Blacks (11.7%) (Centers for Disease Control and Prevention, 2020). Generally, higher body weight increases type 2 diabetes

risk for everyone. Asian Americans are at increased diabetes risk at lower body weight (about 15 pounds lower) than the rest of the general public (Shoily et al., 2021).

Figure 2.2

Prevalence of Type 2 Diabetes in Different Ethnic Groups in the United States of America



Note: Shows prevalence of type 2 diabetes among different ethnic groups in America. Adapted from "Percentage of Adults Aged 18 Years or Older with Diagnosed Diabetes, by Racial or Ethnic Group, United States, 2017–2018" by Centers for Disease Control and Prevention, 2020 (https://www.cdc.gov/diabetes/library/reports/reportcard/national-state-diabetes-trends.html) Copyright 2020 by the Centers for Disease Control and Prevention.

Additionally, type 2 diabetes shows a stronger link to family history than type 1 diabetes. Twin studies revealed that the concordance rate (41-55%) for type 2 diabetes is higher in monozygotic twins relative to dizygotic twins (10-15%). People with positive family history face a 2.4-fold increased risk for developing type 2 diabetes (Matharoo et al., 2017). So far, over 250 genomic regions have been implicated in the predisposition of type 2 diabetes in genome-wide association studies (GWAS) (Langenberg & Lotta, 2018). Genome-wide association and linkage studies found that type 2 diabetes is associated with novel six loci – JAJF1, CDC123CAMK1D, TSPAN8LGR5, THADA, ADAMTS9, and NOTCH2 in European descents, novel six loci (GRB14, ST6GAL1, VPS26A, HMG20A, AP3S2, and HNF4A) in South Asians descents,

eight new loci (GLIS3, PEPD, FITM2 R3HDMLHNF4A, KCNK16, MAEA, GCC1PAX4, PSMD6 and ZFAND3 in East Asian descents (Matharoo et al., 2017). Moreover, single nucleotide polymorphisms (SNPs) (variation at a single position in a DNA sequence among individuals) were found to contribute to the development and progression of diabetes by influencing gene expressions with people from different ethnic backgrounds (Shoily et al., 2021).

Gestational Diabetes

Gestational diabetes is a transient form of diabetes that manifests as hyperglycemia during pregnancy and subsides post-partum. High blood sugar during pregnancy is classified as gestational diabetes. It can occur anytime during pregnancy, most often around 24- or 26-weeks' gestation. About 2-5% of all pregnant women develop gestational diabetes. The placenta provides oxygen and nutrients to the growing fetus during pregnancy and produces estrogen, cortisol, and human placental lactogen hormones, blocking insulin. Those with normal glucose homeostasis can upregulate their insulin production to overcome this problem. However, those with underlying insulin resistance issues cannot overcome this problem, and the extra glucose remains in their bloodstream. This type of hyperglycemia is diagnosed as gestational diabetes (International Diabetes Federation, 2019a; University of Rochester, 2021).

Risk factors for gestational diabetes include older age, overweight and obesity, previous gestational diabetes, family history, and polycystic ovary syndrome. Gestational diabetes usually resolves itself once the pregnancy ends; however, it increases the risk of gestational diabetes in subsequent pregnancies and type 2 diabetes later in life (International Diabetes Federation, 2019a; University of Rochester, 2021). In addition, children and youth of mothers who had gestational diabetes face an increased risk of diabetes themselves (Blotsky et al., 2019). Genome-wide association studies found that a large portion of gestational diabetes can be attributed to

GCK gene mutations, which contribute to abnormal glucose levels during pregnancy and beyond. Other genes associated with gestational diabetes are KCNJ11 and HNF1A, HNF4A (Rosik et al., 2020).

Other Types of Diabetes

These types of diabetes are usually known as secondary diabetes. Many of these are monogenic diabetes, resulting from a single gene defect rather than multiple genes and environmental factors such as type 1, type 2, and gestational diabetes. Four major types of monogenic diabetes are latent autoimmune diabetes in adults, maturity-onset diabetes of the young (MODY), maternally inherited diabetes and deafness, and neonatal diabetes (Prasad & Groop, 2015). Latent autoimmune diabetes of adults is autoimmune diabetes with genetic, immunologic, and metabolic features common with types 1 and 2 diabetes. For instance, this type of diabetes occurs due to autoimmune destruction of beta cells like type 1; however, unlike type 1, the onset occurs around age 35. In addition, with this type of diabetes, the affected individuals do not need insulin therapy at least for the first six months of diagnosis (Rajkumar & Levine, 2020). In 2018, the study entitled “First Genome-Wide Association Study of Latent Autoimmune Diabetes in Adults” found mutations in gene PFKFB3 to be particularly associated with LADA (Chen & Chen, 2019).

Like latent autoimmune diabetes of adults, maturity-onset diabetes of the young (MODY) shares type 1 and type 2 diabetes characteristics. MODY is usually characterized by the onset of insulin resistance and hyperglycemia at an early age, usually before age 25, without any sign of autoimmune destruction of beta cells. It is inherited in an autosomal dominant pattern. A condition can be passed on to children with autosomal dominant mutations when only one parent carries or has the disease gene. So far, 13 genes on different chromosomes have been identified

to be associated with MODY, with the most frequently occurring mutations found to be in GCK, HNF1α, HNF4α, and HNF1β genes (Kleinberger & Pollin, 2015; Urakami, 2019).

Maternally inherited diabetes and deafness (MIDD) is a form of hyperglycemia accompanied by hearing loss (Robinson et al., 2020). Point mutations cause MIDD in three mitochondrial DNA MT-TL1, MT-TK, and MT-TE genes. Because the genes involved with MIDD are in mitochondrial DNA, this condition is inherited in a mitochondrial pattern, also known as maternal inheritance. Only females pass mitochondrial diseases to their children because egg cells, but not sperm cells, contribute mitochondria to the developing embryo. Mitochondrial disorders can appear in every generation of a family. They can affect both males and females, but fathers do not pass mitochondrial traits to their children (National Center for Advancing Translational Sciences, 2021).

Diabetes occurring under six months of age is called “neonatal” or “congenital” diabetes. About 80–85% cases of neonatal diabetes have an underlying monogenic cause. Sometimes, clinicians mistakenly classify neonatal diabetes as type 1 diabetes. However, neonatal diabetes almost always occurs before six months of age, whereas type 1 diabetes usually occurs after six months of age. Neonatal diabetes is either be transient or permanent. The transient form is most often due to an overexpression of genes on chromosome 6q.24, is recurrent in about half of cases, and treatable with medications other than insulin. Permanent neonatal diabetes is most commonly due to autosomal dominant mutations in the genes encoding the Kir6.2 subunit (KCNJ11) and SUR1 subunit (ABCC8) of the b-cell KATP channel. It is crucial to have a correct genetic diagnosis for this type of diabetes as most patients with KATP-related neonatal diabetes can be treated with high-dose oral sulfonylureas instead of insulin (National Center for Advancing Translational Sciences, 2021).

Overall, the other types of diabetes account for only 1.5-2% of all diabetes cases (Gandica et al., 2015; Thomas et al., 2016). This percentage might vary due to misdiagnosis. Monogenic diabetes might be incorrectly diagnosed by the clinicians as either type 1 or type 2 diabetes. Similarly, high blood sugar in adulthood could be latent autoimmune diabetes of adults, and high blood sugar in infancy could be neonatal diabetes (Gandica et al., 2015).

Sometimes diabetes arises due to pancreatic disorders such as pancreatitis, trauma, infection, pancreatic cancer, and pancreatectomy. Additionally, exocrine disorders cause excess secretion of hormones that are insulin-agonists and may increase blood sugar. Certain genetic syndromes such as Prader-Willi syndrome, Down's syndrome, Friedrich's ataxia, Wolfram's syndrome, and cystic fibrosis also mediate the onset of diabetes. Finally, some drugs can disrupt insulin secretion or insulin action and cause high blood sugar (American Diabetes Association, 2019b; Kleinberger & Pollin, 2015).

Regardless of the type of diabetes and the underlying cause, the treatment of diabetes can be difficult. The goal of diabetic treatment is to keep the blood sugar in normal range. A combination of diet and lifestyle modifications and drug therapy is the most common approach. There are numerous medications used in the treatment of diabetes.

Diabetes Medications: Past, Present, and Future

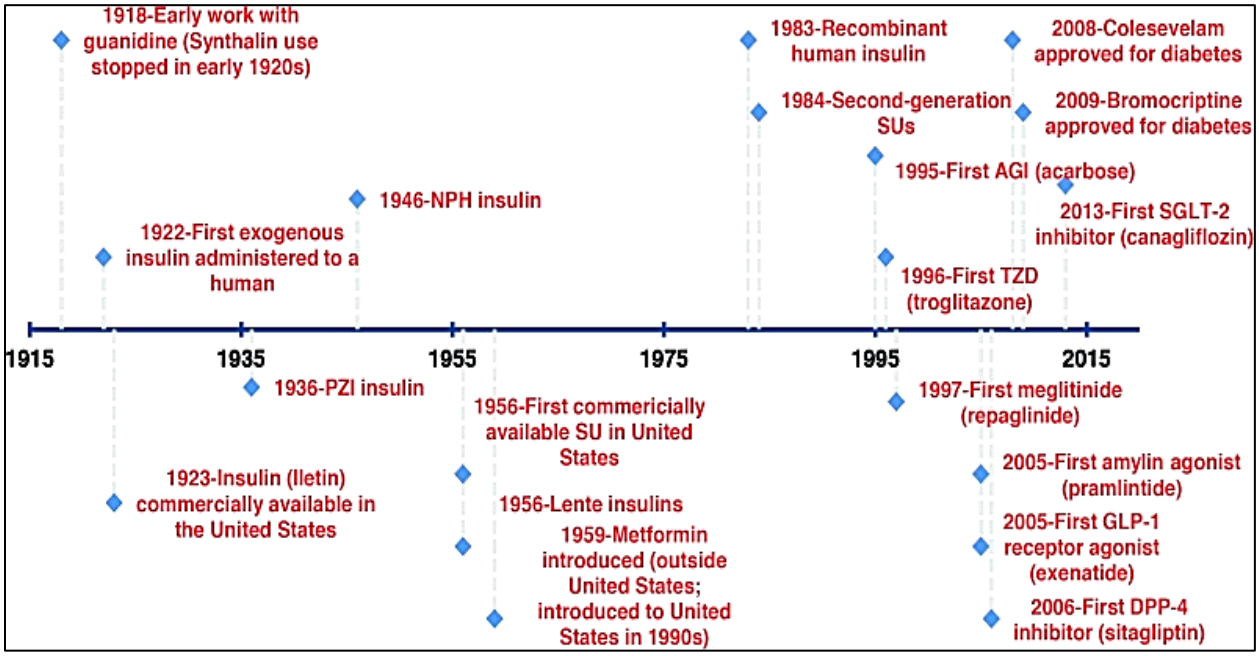
While medical professionals were well-aware of the symptoms of diabetes, nobody knew the cause of the disease until 1889, when German physicians Joseph von Mering and Oscar Minkowski surgically removed pancreases from dogs and discovered that these dogs immediately developed diabetes. This discovery established a link to diabetes and research focused on the pancreas (Karamanou et al., 2016; Tattersall, 2017). In 1921, Dr. Frederick Banting, a Canadian surgeon, and his medical student, Charles Best, successfully extracted

insulin from the pancreatic ducts of different animals and injected dogs without pancreases with this insulin and saw an immediate improvement in their blood sugar levels. Empowered with this information, in 1922, they injected their first diabetic patient with insulin whose blood sugar level showed drastic improvement. For the next 60 years, animal insulin was used to treat diabetes worldwide. Finally, in 1978, recombinant human DNA insulin was invented, which could be synthesized in labs for human use, rendering animal insulin collection unnecessary (Karamanou et al., 2016; Tattersall, 2017)

Since then, steady progress has been made in drug developments for diabetes, especially for non-insulin-dependent patients. Figure 2.3 shows the timeline for different diabetes medications. The most common diabetes medication, biguanides, known as metformin, was introduced worldwide in 1959 and approved in the United States in the 1990s. Biguanides reduce blood sugar by reducing hepatic glucose production and increasing cellular blood sugar uptake. Thiazolidinediones (TZDs), or Glitazones, reduce blood sugar similarly by reducing hepatic glucose production and increasing skeletal muscle insulin sensitivity. These drugs were introduced in the U.S. market in the 1990s, and are still prescribed despite an increased risk of certain adverse effects such as bone fractures, bladder cancer, and congestive heart failures (National Center for Biotechnology Information, 2021; White, 2014).

Figure 2.3

Development of Diabetes Medications



Note: Shows development of diabetes medications from 1915 to 2015. Adapted from "A brief history of the development of diabetes medications" by White, 2014 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4522877/). Copyright 2014 by John R. White, JR.

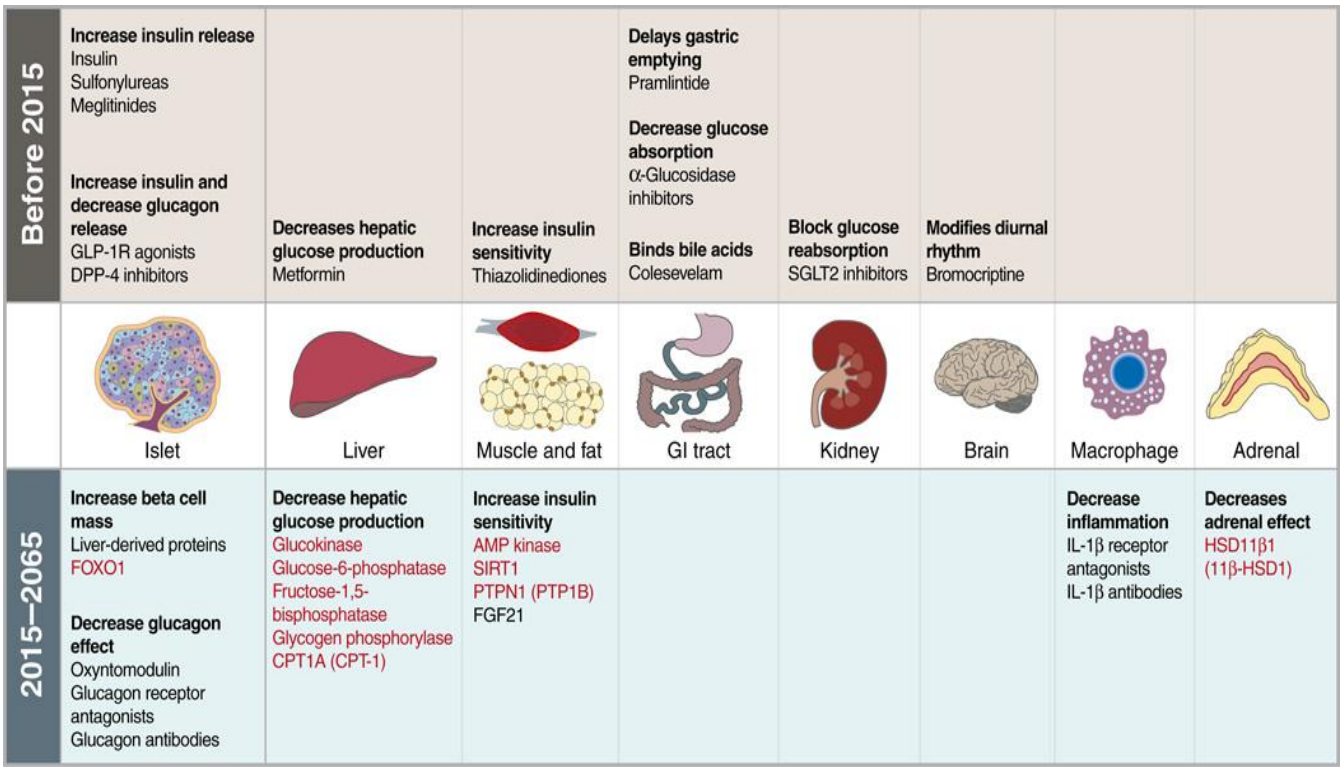
Another frequently used medication for diabetes, sulfonylureas, were marketed in Germany in the 1950s and in the United States around the 1980s. Sulfonylureas work by increasing pancreatic beta-cell activity and insulin production. The meglitinides or glinides are structurally different from sulfonylureas but work similarly by increasing beta-cell activity and insulin production. Meglitinides became available in the United States in the early 2000s. Glucagon-like Peptide-1 receptor agonists were approved for use in the early and mid-2000s. They work on the insulin-incretin pathway by increasing insulin hormone secretion and blocking glucagon production by the alpha cells. This class of medications is administered subcutaneously. DPP-4 inhibitors work similarly but could be taken orally as well. They were approved for use in the mid-2000s (White, 2014).

Less commonly used diabetes medications include alpha-glucosidase inhibitors, amylin agonists, bromocriptine and Colesevelam, and sodium-glucose co-transporter-two inhibitors. Alpha-glucosidase inhibitors have been available in the United States since the 1990s. They work by inhibiting alpha-glucosidase enzymes responsible for the breakdown of oligosaccharides, trisaccharides, and disaccharides, reducing overall carbohydrate absorption. Amylin agonists, also approved around the 1990s, inhibit glucagon secretion. Bromocriptine is a dopamine agonist found to decrease blood sugar in diabetes and therefore approved for use in 2009. However, the mechanism of action remains unclear. Similarly, Colesevelam, a bile acid sequestrant, was initially used as a cholesterol-lowering medication found to lower blood sugar in patients; therefore, it was approved as diabetes medication in 2010. Finally, sodium-glucose co-transporter-2 inhibitors (SGL2) were approved around 2015, which works by inhibiting glucose reabsorption by the kidneys (White, 2014).

While all these medications help the patients manage diabetes, they are not personalized. Therefore, even with drugs, people with diabetes experience bouts of hypo and hyperglycemia. Consequently, they face various degrees of macro and microvascular complications of diabetes such as cardiovascular disease, chronic kidney disease, and feet and vision problems in the long run. Thus, current research focuses on personalized medicine for diabetes to reduce these complications (Kahn et al., 2015).

Figure 2.4

Diabetes Medications: Past, Present, and Future



Note: Shows past, present, and future diabetes medications. Adapted from "Medications for type 2 diabetes: how will we treat patients in 50 years" by Kahn et al., 2015 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4499484/) Copyright 15 by Kahn et al.

Figure 2.4 illustrates the novel research that is taking place for diabetes. A better understanding of the pathogenesis of diabetes at a cellular and genetic level is driving the investigation for novel and personalized therapeutic agents for diabetes. The figure divides the types of diabetes treatments before 2015 and after 2015. Before 2015, research on diabetic medications focused on different organ systems to have glucose-lowering effects. Since 2015, research on diabetes medicine has focused on cellular and genetic levels of those organ systems such as carnitine palmitoyltransferase 1A (CPT1A), fibroblast growth factor 21 (FGF21), forkhead box protein O1 (FOXO1), gastrointestinal (GI), 11β-hydroxysteroid dehydrogenase

type 1 (HSD11β1), sirtuin 1 (SIRT1), protein tyrosine phosphatase (PTPN1) and non-receptor type 1 (Kahn et al., 2015).

Gene Therapy for Diabetes

Gene therapy is a technique that repairs or reconstructs defective genetic material to treat or prevent disease. As discussed earlier, all forms of diabetes have genetic components that increase this disease's susceptibility (American Diabetes Association, 2021b). Thus, gene therapy has the potential to treat diabetes successfully. To understand gene therapy for diabetes, it is essential to understand the mechanisms of gene therapy. Therefore, in this section, the means of gene therapy will be discussed.

The Flow of Genetic Information

It is essential to learn how gene expression is regulated to understand gene therapy fully. The central dogma explains this phenomenon, stating that the genes are sequences of DNA that code for functional proteins. The Central Dogma, the guiding principle of gene regulation, was first proposed in 1958 by Francis Crick, the discoverer of DNA structure. Central dogma essentially states that genetic flow in humans is one-directional; the genetic codes in the DNAs are transcribed into RNAs which then translated into functional proteins (Northeastern University, n.d.).

DNA ➡ RNA ➡ Protein

Insulin is a functional protein (polypeptide) (Colorado State University, n.d.). Any mutation to the DNA that codes for insulin can affect insulin production. As all diabetes is genetic, it is clear that there are mutations in the human DNA that are causing this disease (American Diabetes Association, 2021b). Unfortunately, as the gene flow in humans is essentially one-directional, a human body cannot undo the genetic mutations. However,

microorganisms might help with this process. Certain viruses such the RNA viruses and retroviruses can go from DNA to RNA or RNA to DNA (Northeastern University, n.d.).

DNA  RNA  Protein

Therefore, this mechanism is manipulated to create viral vectors that can help correct genetic mutations in humans. Additionally, the CRISPR-Cas9 system, a genome-editing tool, has been created in 2011 from certain types of bacteria such as *Streptococcus pyogenes.* This system helps find mutated genes and edit them. This system consists of two RNAs; one guides the enzyme Cas9 to find the defective DNA and cut it, and the other works as a repair template with correct codes used to fix the genome (National Library of Medicine, 2020). These inventions were created with the Central Dogma in mind in order to fix the genetic mutations by reversing the flow of genetic flow of information.

Genetic Engineering and Gene Therapy

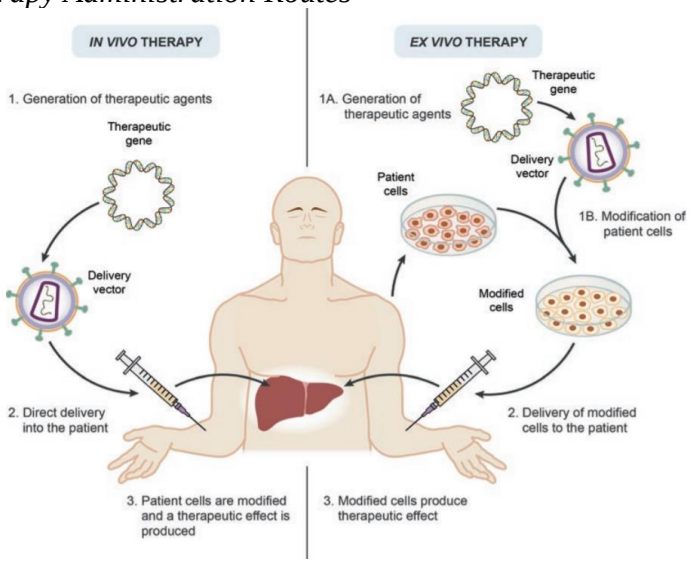
Humans indirectly controlled the genomes of plants and animals for centuries with selective breeding. Genetic engineering presents opportunities to directly manipulate one or more genes to receive the desired phenotype where selective breeding is no longer required. When genetic engineering is utilized to introduce, remove, or change a person's genetic code to prevent, treat, or cure a genetic disorder, it is called gene therapy. With this, a disease-causing protein formation could be changed in a person to prevent, treat, or cure a disease in a particular person (American Society of Gene and Cell Therapy, 2021b).

Generally, genetic engineering is administered through two different modes: germline or somatic. With germline engineering, genetic modifications are performed on reproductive cells (eggs or sperm) to make the traits heritable to future generations. This way, a hereditary disorder could be removed from a family line forever (National Human Genome Research Institute, 2021;

Tamura & Toda, 2020). On the other hand, with somatic genetic engineering, the manipulated genes only affect the individual, without passing them down to the next generation. As illustrated in Figure 2.5, somatic gene transfer occurs in two significant ways: in-vivo or in-vitro. With in-vivo transfers, the transgene should be appropriately directed to the target cells, and the gene products should be protected from immune attacks. With in-vitro transfers, it is crucial to properly remove target cells and transplant them back into the host (National Human Genome Research Institute, 2021; Tamura & Toda, 2020).

Figure 2.5

Gene Therapy Administration Routes



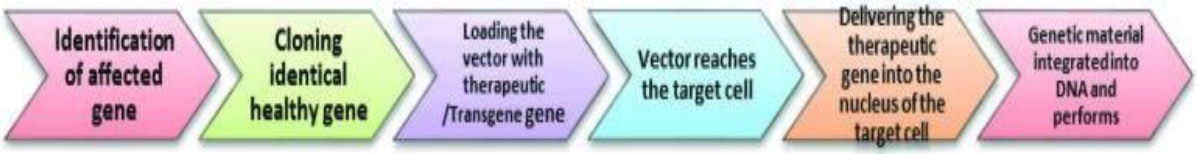
Note: Illustrates in-vivo and ex-vivo gene therapy. Adapted from "Handbook of Cell and GeneTherapy" by Nobrega et al., 2020. Copyright 2020 by Nobrega et al.

Regardless of the administration route, the most critical first step for gene therapy is identifying the faulty gene. These defective genes could be placed in different ways by analyzing patterns of inheritance of disease, studying the metabolisms of the patients who have the disease, and analyzing the genes of the patients who have the disease. The next step is to identify the corresponding normal gene. Once this is determined, it needs to be isolated and copied. Making multiple copies of a single gene is called "cloning." For the cloning process to occur, the DNA

strand of the gene of interest is inserted into bacteria or yeast to allow rapid replication. This part of the process is called recombinant DNA technology, joining DNA molecules from two different species. As the bacteria or yeast cells proliferate (multiply), the DNA strand gets replicated. These copies of DNA strands can then be purified from other cell components, and the genes of interest could be cut away from the unwanted DNA sequence. These copies of the gene of interest now could be combined with DNA suitable for insertion into human cells. These genes are now called therapeutic genes or transgenes. These are then loaded in a vehicle called a vector whose function is to deliver the therapeutic gene to the patient's target cell. After the vector reaches the target cell, it delivers the genetic material to the nucleus. The genetic material gets integrated into DNA in the nucleus and corrects the defective or mutated gene. Figure 2.6 illustrates key steps in gene therapy (National Human Genome Research Institute, 2021; Tamura & Toda, 2020).

Figure 2.6

Process of Gene Therapy



Note: Shows steps in gene therapy. Adapted from "Non-viral vectors in gene therapy" by Wang & Gao, 2015 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4347098/). Copyright 2015 by Wang & Gao.

Gene Delivery System

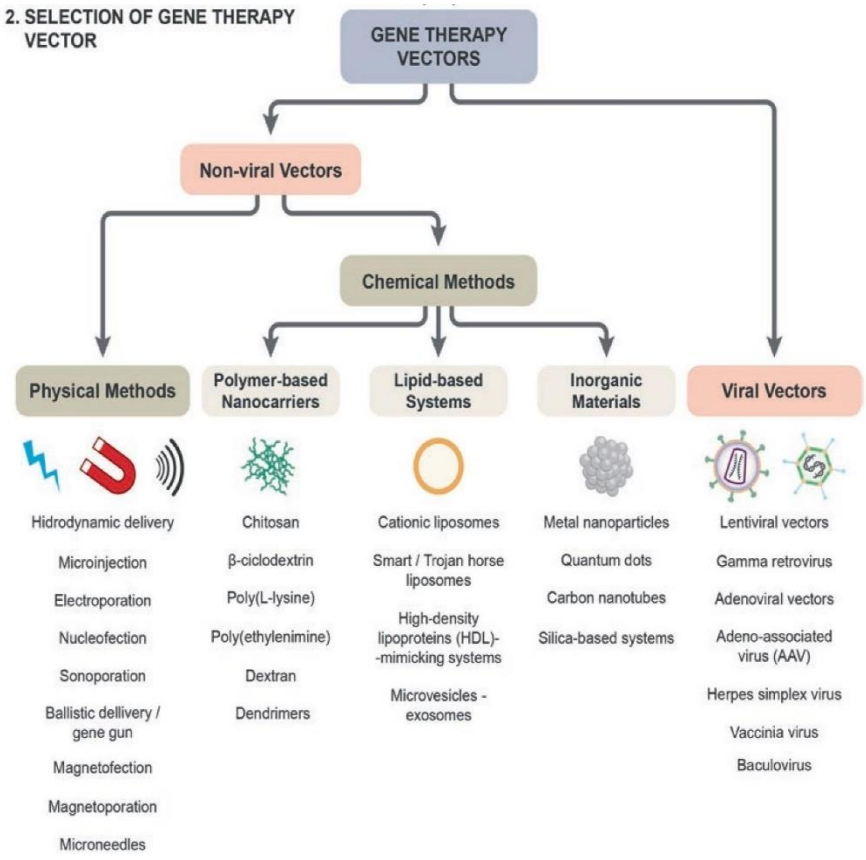
Most organisms and cells block outside genetic materials, making gene delivery difficult. Therefore, the delivery of the exact therapeutic sequence needs careful consideration for successful gene therapy. The choice of the correct/ideal delivery system for given gene therapy is dependent on many factors, such as the size of the gene, the expected effect, and the toxicity

profile. Currently, there are two delivery systems available for gene therapy, the viral and the non-viral methods. The viral systems take advantage of the broad diversity of viruses and their innate ability to infect/transduce cells. The main advantage of these systems is their high efficiency, and the main disadvantage is the safety concerns of using modified viruses (Nóbrega et al., 2020).

On the other hand, non-viral systems are safer but less efficient (Nóbrega et al., 2020). The non-viral vectors are further subdivided into two categories: the physical and chemical methods. Both methods are illustrated in Figure 2.7. Physical methods include hydrodynamic delivery, microinjection, electroporation, nucleofection, sonoporation, gene gun, magnetoreception, magnet operation, and microneedles. In contrast, chemical vectors include polymeric-based systems, lipid-based systems, metal nanoparticles, quantum dots, and graphene-based systems such as carbon nanotubes and silica nanoparticles (Nóbrega et al., 2020).

Figure 2.7

Gene Therapy Vectors



Note: Shows different types of vectors used in gene therapy. Adapted from "Handbook of Cell and Gene Therapy," by Nobrega et al., 2020. Copyright 2020 by Nobrega et al.

Viral vectors are the most frequently used vehicles for gene therapy. So far, nearly 68% of clinical trials have used viral vectors as their delivery system. The viral genes are removed first with viral vectors, so only the therapeutic genes are delivered. Researchers carefully choose which viral vector to use to treat a disease based on how well researchers understand the virus, how well it can target specific cells, and how safe it is to use. There are four main types of viral vectors available: adeno-associated viral (AAV) vectors, adenoviral vectors, adeno-associated viral (AAV), lentiviral and retroviral vectors. Adeno-associated viral vectors (AAV) are the most

used viral vectors. They can deliver only small DNA packages, or genes, to cells but do not insert themselves into a cells' genome (Nóbrega et al., 2020).

Additionally, they do not usually induce adverse immune reactions are most people are exposed to these viruses through natural infections. Adenoviral Vectors can deliver packages up to eight times larger than AAVs; however, they tend to cause strong immune responses in humans. Lentiviral and Retroviral Vectors can deliver larger genetic packages of RNA, which is then converted into DNA (American Society of Gene and Cell Therapy, 2021b)

Gene Therapy Strategies

Gene therapy is performed through strategies that modify the expression of an individual's genes or correct abnormal genes. Each strategy involves the administration of a specific DNA or RNA through gene addition, gene correction, gene silencing or shutdown, reprogramming, cell elimination, and combined gene and cell therapy. These strategies are explained in detail in below (American Society of Gene and Cell Therapy, 2021b; Nóbrega et al., 2020).

Gene Addition. This process is also called gene augmentation. With this approach, a new protein-coding gene is inserted into the target cells to produce more of the desired protein. Gene addition is considered the most straightforward form of gene therapy. Usually, viral vectors, such as adeno-associated viruses (AAV), are utilized to deliver the new gene into the target cells. This technique has been used to treat various diseases, such as severe combined immunodeficiency, congenital blindness, hemophilia, Leber's congenital amaurosis, lysosomal storage diseases, X- and linked chronic granulomatous disease (American Society of Gene and Cell Therapy, 2021b; Nóbrega et al., 2020).

Gene Correction or Gene Editing. This approach is used to remove repeated or faulty elements of a gene or replace a damaged or dysfunctional region of DNA using gene-editing technologies such as CRISPR/cas9, TALEN, or ZFN. It is crucial to identify the specific region of the genome to be altered and create conditions to produce a protein that functions normally instead of in a way that contributes to disease. For example, Duchenne muscular atrophy (DMD) is a hereditary disorder that arises because of mutations in the DMD gene. The DMD gene codifies dystrophin in healthy individuals that plays a crucial role in muscular structure and physiology. CRISPR/Cas9 tools effectively fixed dogs with the defective DMD gene, improving dystrophin levels and muscular physiology (American Society of Gene and Cell Therapy, 2021b; Nóbrega et al., 2020).

Gene Silencing or Shutdown. This technique is exploited for dominantly inherited disorders where a single abnormal gene is sufficient to cause a certain disease. Therefore, silencing this gene can prevent the disease from manifestation. In this type of gene therapy, abnormal protein production by the faulty gene is prevented by targeting and degrading the messenger RNA (mRNA; an intermediate required for protein expression from a gene). mRNA exists in a single-stranded form in human and animal cells, whereas some viruses (class III) have double-stranded RNA. Human and animal cells recognize double-stranded RNA as being viral in origin and destroy it to prevent its spread. Therefore, gene silencing technique uses small RNA sequences to bind unique sequences in the target mRNA and make it double-stranded, triggering the mRNA's destruction using the cellular machinery that destroys viral RNA. Currently, gene silencing is being employed to treat tumor necrosis factor (TNF) alpha in rheumatoid arthritis patients (American Society of Gene and Cell Therapy, 2021b; Nóbrega et al., 2020).

Reprogramming. This technique changes the characteristics of a specific cell. Sometimes, a disease is caused by a dysfunction of a specific type of cell where multiple cell types exist. For example, diabetes occurs because many of the pancreas' insulin-producing beta cells are damaged while the other cells such as alpha and delta cells are intact. Therefore, reprogramming the alpha or delta cells to produce insulin could help diabetic patients (American Society of Gene and Cell Therapy, 2021b; Nóbrega et al., 2020).

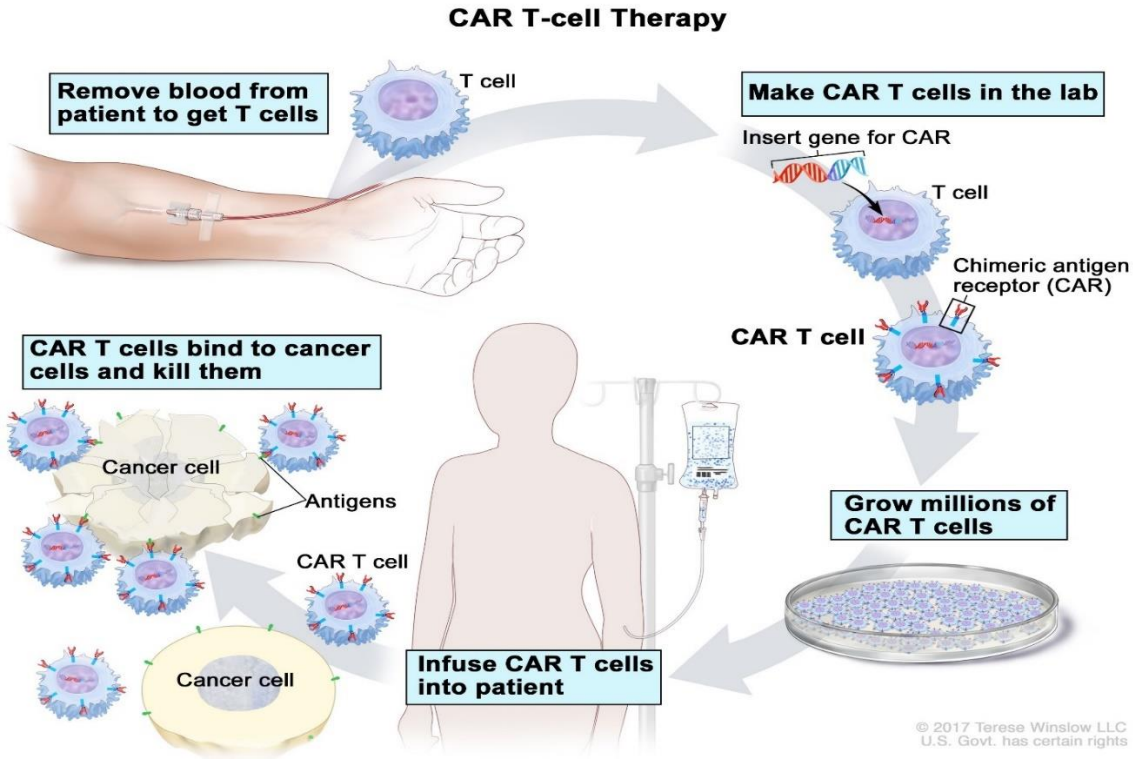
Cell Elimination. This method is sometimes called suicide gene therapy**.** This strategy is utilized to destroy malignant (cancerous) tumor cells and eliminate benign (non-cancerous) tumor cells by introducing "suicide genes," which enter the tumor cells and release a substance that induces apoptosis (cell death) in those cells. Viruses are engineered to have an affinity for tumor and carry suicide genes to increase toxicity to tumor cells, then stimulate the immune system's attack on the tumor (American Society of Gene and Cell Therapy, 2021b; Nóbrega et al., 2020).

Combined Gene and Cell Therapy. Sometimes, gene therapy is combined with cell therapy. Cell therapy is different from gene therapy. Cell therapy refers to a process where live and intact cells are transferred to a person to prevent, treat, or cure disease instead of manipulating a person's genetic code. A common type of cell therapy is blood transfusion, where red blood cells, white blood cells, and platelets from a donor are transfused into a recipient. This cell therapy could be taken one step further and change the genes in those cells to have the desired effect by combining cell and gene therapy. For example, CAR (chimeric antigen receptor) T-cell uses gene and cell therapy to treat blood cancers. With this type of treatment, a patient's T cells (a type of immune system cell) are taken from them and genetically modified in a laboratory to attack cancer cells and returned to them by infusion. Figure 2.8 illustrates the

process (American Society of Gene and Cell Therapy, 2021b; National Cancer Institute, 2021). The United States Food and Drug Administration (US FDA) has approved CAR-T therapy to treat aggressive B-cell lymphomas in adults, B-cell leukemia in children and young adults, and relapsed or refractory mantle cell lymphoma (MCL) in adults (American Society of Gene and Cell Therapy, 2021a).

Figure 2.8

CAR-T Cell Therapy



Note: Illustrates cell and gene therapy combined. Adapted from "CAR-T cell Therapy" by American Cancer Society (https://www.cancer.gov/publications/dictionaries/cancer-terms/def/car-t-cell-therapy). Copyright 2020 by American Cancer Society.

The cells used in cell therapy can originate from the patient (autologous cells) or a donor (allogeneic cells). The cells used in cell therapy are classified by their potential to transform into different cell types, a process called cell differentiation. A totipotent cell type can differentiate

into any cell type, and the cell type called multipotent cells can differentiate into a few different types of other cells. The cells that have already been differentiated are a fixed cell type and cannot be transformed into any other cell type. The kind of treatment determines which type of cell will be used for cell therapy (American Society of Gene and Cell Therapy, 2021b).

Worldwide Approved Gene Therapy Products to Date

The idea of gene therapies first came about in the 1960s with the development of recombinant DNA (rDNA) technology. Genes encoding human insulin and growth hormone were cloned and expressed in E. coli in 1978 and 1979 respectively. The first licensed drug produced using recombinant DNA technology was human insulin, called Humulin, developed by Genentech and licensed and marketed by Eli Lilly in 1982. With further research, in 1990, the first gene therapy clinical trial took place in America at the National Institutes of Health in Bethesda, Maryland. Two patients, Ashanthi DeSilva and Cindy Kisik, participated in this trial. Both patients suffered from adenosine deaminase (ADA) deficiency, a monogenic condition that causes severe immunodeficiency. In this trial, they were given functional copies of the gene encoding the ADA enzyme, which improved their immune systems and allowed them to live normal lives without being in isolation to avoid constant infection. This event marked a significant milestone for gene therapy, and many clinical trials started at this time (American Society of Gene and Cell Therapy, 2021a; Nóbrega et al., 2020; Tamura & Toda, 2020).

However, this field hit a major setback in1999 with the death of an 18-year-old patient, Jesse Gelsinger, who volunteered for an experimental gene therapy trial at the University of Pennsylvania. Jesse suffered from a genetic condition known as ornithine transcarbamylase deficiency, which reduced his liver’s ability to break down toxic ammonia accumulated in his body. Jesse received a functional copy of the gene delivered by an adenovirus vector in this trial.

Unfortunately, Jesse developed a reaction to this vector and died four days later causing a media uproar. However, the other 17 patients in the trial only had mild adverse effects and showed overall improvement with the therapy (American Society of Gene and Cell Therapy, 2021a; Nóbrega et al., 2020; Tamura & Toda, 2020).

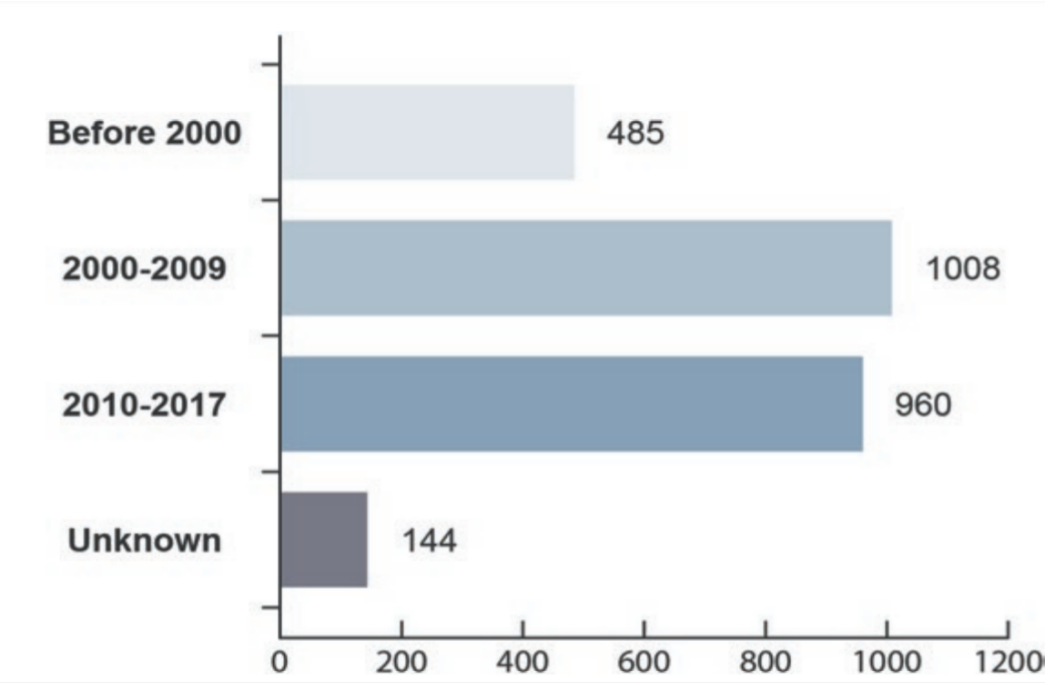
Nevertheless, the U.S. FDA suspended the university's entire gene therapy program in addition to launching investigations in the 69 other ongoing trials at the time. To make matters worse, in 2002, four patients developed leukemia, and one died after 60 months after a clinical trial that treated 10 boys with X-linked severe combined immunodeficiency (X-SCID) in Europe. Investigations conducted after the trial revealed that the therapeutic transgene was inserted near an oncogene, which caused cancer in those patients. Interestingly, a 10-year follow-up with the surviving patients showed that the gene therapy corrected the X-SCID mutation in those patients (American Society of Gene and Cell Therapy, 2021a; Nóbrega et al., 2020; Tamura & Toda, 2020).

While the U.S. FDA continued suspensions and moratoriums on all gene therapy clinical trials citing safety concerns in the United States of America and Europe, other countries moved forward with genetic research. China became the first nation to approve a gene therapy product in 2003 for head and neck cancer, called Genedicine. Russia approved the next gene therapy product for peripheral artery disease named Neovasculgen in 2011. The European Commission approved Glybera for an ultra-rare disease lipoprotein lipase deficiency in 2012. In 2018, the European Commission approved another gene therapy product named Luxturna for genetic mutations that cause blindness. Finally, in 2019, Zolgensma was approved by the U.S. FDA for genetic mutations that cause spinal muscular dystrophy (American Society of Gene and Cell Therapy, 2021a; Nóbrega et al., 2020; Tamura & Toda, 2020).

Despite the rocky start, the interest in this field prevailed, and research continued. With further research, knowledge and techniques for gene therapy improved. Additionally, important discoveries and advances like the Human Genome Project, stem cell research, improvements in vectors, and gene editing techniques like TALENs or CRISPR provided more promising ways to conduct gene therapy (Nóbrega et al., 2020).

Figure 2.9

Number of Gene Therapy Clinical Trials Over the Years



Note: Shows number of gene therapy clinical trials from 2000 to 2017. Adapted from "Handbook of Cell and Gene Therapy" by Nobrega et al., 2020. Copyright 2020 by Nobrega et al.

As illustrated in Figure 2.9, over 2500 clinical trials took place worldwide by the end of 2017. About 65% of all attempts were on cancer, 11% were on monogenic diseases, and 7% were on cardiovascular diseases. However, with strict regulations placed on clinical trials by different regulatory agencies, especially the U.S. FDA and the European Commission, only 22 gene therapy products have been approved by the end of 2019. Table 2.1 summarizes the

approved gene therapy products and their indications. Eight of these products were in the monogenic disorder category, six in the cancers category, four to treat cardiovascular diseases, one to treat infectious diseases, one to treat degenerative arthritis category, one for ocular diseases, and the last one was from the other category (Ginn et al., 2018; Ma et al., 2020; Nóbrega et al., 2020). Only one of these products, Neovasculgen, could be used for diabetes-related foot problems (Ma et al., 2020). However, no gene therapy products to treat diabetes in humans have been approved thus far.

Table 2.1

Approved Gene Therapy Products from 1998 to 2019	
Names	**Indications**
Zynteglo	β-thalassemia
Zolgensma	Spinal muscular atrophy
Waylivra	Familial chylomicronemia syndrome
Collategene	Critical Limb Ischemia
Onpattro	Amyloidosis
Tegsedi	Amyloidosis
Luxturna	Biallelic RPE65 mutation-associated retinal dystrophy
Yescarta	Large B-cell lymphoma
Kymriah	B-cell precursor acute lymphoblastic leukemia and large B-cell lymphoma
Invossa	Knee osteoarthritis
Spinraza	Spinal muscular atrophy
Exondys 51	Duchenne muscular dystrophy
Zalmoxis	Restore s immune system HSCT transplant
Strimvelis	ADA-SCID
Imlygic	Melanoma
Kynamro	Homozygous familial hypercholesterolemia
Glybera	Familial lipoprotein lipase deficiency and pancreatitis attacks
Neovasculgen	Atherosclerotic peripheral arterial disease, including critical limb ischemia
Rexin-G	Soft tissue sarcoma, osteosarcoma, and pancreatic cancer
Oncorine	Nasopharyngeal cancer
Gendicine	Head and neck cancer
Vitravene	Cytomegalovirus retinitis

Note: adapted and modified from “The approved gene therapy products worldwide: from 1998 to 2019” by Wang, et al., 2020 (https://www.sciencedirect.com/science/article/abs/pii/S0734975019302022) Copyright 2020 by from Wang, et al.

Gene Therapy for Diabetes

The idea of gene therapies to treat diabetes first came onboard with recombinant DNA technology, which was used to produce the first synthetic human insulin, “Humulin,” in 1978. With recombinant DNA technology, a human insulin gene is inserted into another organism’s genome, usually an E. coli bacterium enabling this “recombinant” bacterium to produce the insulin protein encoded by the human insulin gene. Then the insulin is harvested and purified for human (Baeshen et al., 2014). In 1983, Fineberg et al. (1983) published the results of a study in 221 individuals with diabetes who had undergone 12 months of insulin treatment showing that patients treated with synthetic human insulin had lower levels of insulin antibodies in blood than patients treated with porcine insulin. The findings indicated that the new synthetic human insulin was better tolerated by humans than animal-derived insulin (Fineberg et al., 1983).

Additionally, recombinant DNA technology was used to create a human glucagon like peptide (GLP-1), “Victoza,” created by Novo Nordisk and approved by the FDA in 2010 (U.S. Food and Drug Administration, 2019b). GLP-1 is an incretin hormone secreted from intestinal L-cells that stimulates insulin gene expression and insulin biosynthesis, which increases insulin secretion, increases insulin sensitivity, suppresses glucagon secretion, promotes beta cell proliferation, and restores beta cell function in non-responsive beta cells. However, like any other peptides, GLP-1 needed to be administered subcutaneously, not orally, as they are rapidly deactivated gastrointestinal enzymes. Unfortunately, the prospect of daily injections reduced patient interest and compliance for Victoza. Fortunately, Novo Nordisk came up with an oral formulation of GLP-1 agonist with specialized encapsulation that can bypass the effects of some

gastrointestinal enzymes and exert the same effects as the injections. The FDA approved this drug, called “Rybelsus,” in 2019 as this brought down HbA1c levels to less than 7% in type 2 diabetes patients in several clinical trials, thereby proving its efficacy in blood sugar management (Anderson et al., 2020; U.S. Food and Drug Administration, 2019a).

While insulin and GLP-1 agonists are useful in managing blood sugar for diabetes, they do not offer permanent solutions to the problem. Therefore, search for permanent solution is ongoing. Some patients receive whole pancreatic transplantion to normalize blood sugar levels. However, due to significant risks and expenses that come with total pancreatic transplantation, the prospect of islet transplantation is being pursued. An experimental treatment for type 1 diabetes, pancreatic islet transplantation, sponsored by the National Institutes of Health, is currently in phase 3 clinical trial (National Institutes of Health, 2021). In this process, called the Edmonton Protocol, islets with healthy beta cells are collected from the pancreas of a deceased organ donor and then injected into a vein that carries blood to the liver of a person with type 1 diabetes, as illustrated in Figure 2.10. The islets are injected into the liver instead of the pancreas because the liver can regenerate cells and create new blood vessels for these transplanted cells (De Klerk & Hebrok, 2021; National Institutes of Health, 2021).

Figure 2.10

Pancreatic Islet Transplantation Into a Donor's Liver

Note: Illustrates pancreatic islet transplantation in liver that produces insulin ectopically. Adapted from "The islets are transplanted into the recipient's liver" by National Institutes of Health, 2021 (https://www.niddk.nih.gov/health-information/diabetes/overview/insulin-medicines-treatments/pancreatic-islet-transplantation) Copyright 2021 by the National Institutes of Health.

It takes about two weeks after the injection for new blood vessels to form and connect the islets with the existing blood vessels of the patient. These islets then begin to make and release insulin in the patient's body. During the clinical trials, nearly nine out of 10 transplant recipients had HbA1C levels below 7% one year after transplantation. Additionally, five out of 10 patients no longer needed exogenous insulin one year after transplantation (National Institutes of Health, 2021; Voglová et al., 2017). Unfortunately, with any transplantation, the patients need to be on lifelong immunosuppressant drugs so that the donor cells are not rejected by their bodies. One other problem is that about 400,000 working beta cells are needed to be transplanted to mimic a functional pancreas which is challenging to find in one deceased donor (National Institutes of Health, 2021). Therefore, multiple donors are needed to complete one islet transplantation. In 2017, there were only 31,812 organ donors globally, while the number of people with diabetes was 422 million. Therefore, with multiple donors and some failed islet transplantations, only

0.001% of the world's diabetic population could be treated per year with this method (Shapiro, 2018). Additionally, sometimes patients experience adverse events from these transplantations such as peritoneal hemorrhage, hepatic hematoma or hemorrhage, portal vein thrombosis, abnormal liver function, mucosal inflammation, pneumonia, increased blood creatinine, renal disorder, skin disorder, hypertension, and immunosuppression related disorder called leukopenia (De Klerk & Hebrok, 2021).

Therefore, a more efficient way to restore beta cell function is still being pursued. Porcine (pig) islets are suitable for transplantation in humans as porcine beta cells have a similar "set point" for insulin production and are relatively safe for humans. In addition, porcine islets can be genetically modified to reduce the antigen, galactose-alpha-1,3-galactose (Gal epitope), to reduce immunogenic reaction and immune rejection from recipients. Additionally, gene-editing technology, zinc-finger nucleases are being used to knock out the interleukin-2 receptor gamma (IL2RG) gene and to reduce the risk for blood clots and safer insulin-producing porcine cells for grafting. So far, non-human primate studies have shown some success with porcine islet transplants with an anti-CD154 monoclonal antibody regimen. Furthermore, another gene-editing technology, CRISPR-Cas9 is being used to replace large segments of the rat genome with a corresponding human sequence that can generate cells that could be used for insulin production suitable for humans (Bellin & Dunn, 2020).

Moreover, islet cells are being genetically engineered to have enclosing or microencapsulation to stop the recipient's body from rejecting these cells (Bellin & Dunn, 2020). So far, encapsulated pancreatic endoderm cells, differentiated from human embryonic stem cells (ESCs), have already entered the first clinical trials NCT03162926 (completed), NCT03163511

(recruiting), NCT02239354 (active, not recruiting), and NCT02939118 (enrolling by invitation) (ClinicalTrials.gov, 2021b).

Table 2.2

Advantages and Disadvantages of Different Kinds of Islet Transplantations

Current state of pancreas, human islet and porcine islet transplantation and considerations

Transplantation approach	Clinical accessibility	Advantages	Disadvantages	Therapeutic goals
Pancreas transplantation	Clinical procedure	High potential for insulin independence with single transplant	Major surgical procedure; increased risk of surgical morbidity Limited supply	Insulin independence (primary) Eliminate hypoglycaemia (primary or secondary)
Human islet transplantation	Research (USA); clinical (selected regions in Canada, Europe and elsewhere)	Minor procedure Potential for encapsulation	Delayed onset of function >1 transplant may be needed for insulin independence Limited supply	Eliminate hypoglycaemia (primary) Insulin independence (secondary)
Porcine islets	Research in non-human primates; more data needed for human studies	Unlimited islet supply Potential for genetic engineering Potential for encapsulation	More challenging to immunoprotect xenoislets Increased safety concerns (xenotic infection, PERV)	To be determined

Note: Shows advantages and disadvantages of different kinds of transplantation. Adapted from "The current state of the pancreas, human islet, and porcine islet transplantation and consideration" by Bellin and Dunn, 2020 (https://link.springer.com/article/10.1007/s00125-020-05184-7). Copyright 2020 by Bellin and Dunn.

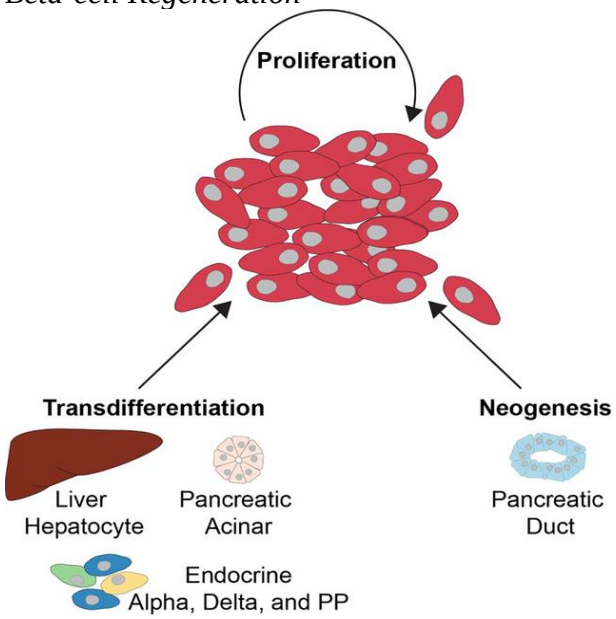
Table 2.2 lists different types of transplantation and considerations. While total pancreatic transplantation can offer complete freedom from daily insulin injections, it comes

with high risk of surgical mordibity and limited supply. Human islet transplantation is more affordable than total pancreatic transplantation, however supply is still limited. However, porcine islets are unlimited in supply, offers the potential for genetic modification and encapsulation to avoid negative immune response and therefore make good candidates for trasplantation (Bellin & Dunn, 2020).

Some other research is taking place in pancreatic cellular regeneration to treat diabetres. Cellular regeneration occurs mainly in three different ways: 1) proliferation of beta cells, 2) neogenesis of beta cells from other cells, 3) transdifferentiation of beta cells from other cell types. All three processes can be performed either *in vivo* or *in vitro* (Guney et al., 2020). As illustrated in Figure 2.11, pancreatic beta cells can be stimulated to proliferate, pancreatic duct cells can be induced to create a new source of beta cells, and pancreatic acinar cells such alpha cells can be genetically reprogrammed into functional beta cells (Guney et al., 2020).

Figure 2.11

Beta-cell Regeneration



Note: Shows different ways beta cell is regenerated. Adapted from"Three mechanisms of beta cell regeneration" by Guney et al., 2020

(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7454996/)n Copyright 2020 by Guney et al.

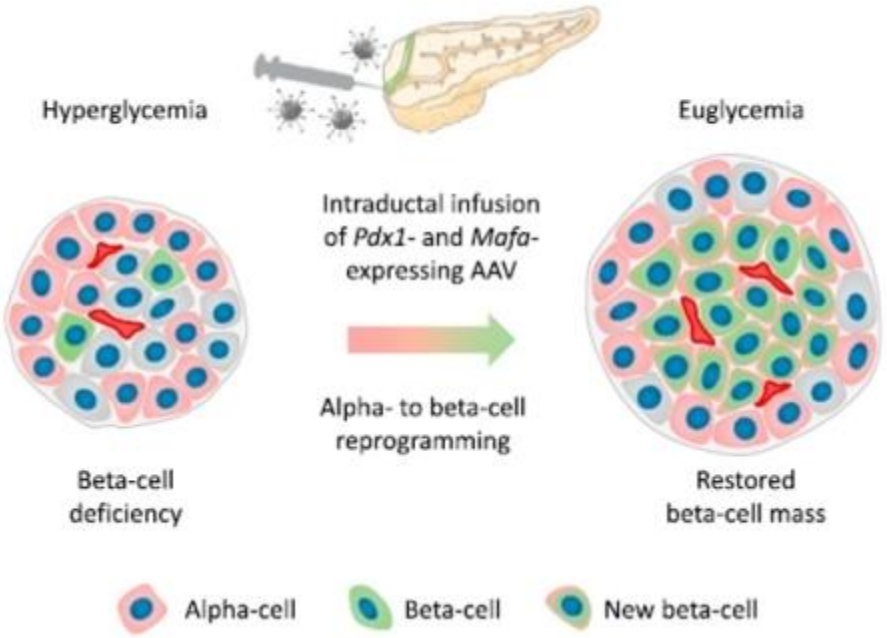
In humans, beta cells proliferate actively during embryonic development and the first years of infancy and then subside. The only other time beta cells proliferate in adults is during turmor (teremed insulinomas) formation. Unfortunately, this happens uncontrollably and harmfully. There has been some interest in increasing beta-cell mass in adults with appropriate stimualtion and without inducing cancer. Certain substances such as harmine, 5-iodo-tubericidin (5-IT), INDY, and GNF4877 inhibit dual-specificity tyrosine phosphorylation–regulated kinase 1A (DYRK1A) shown to increase beta-cell proliferation *in vitro* human cells. However, it was found to that these substances can increase cell proliferation in other cells as well, making it difficult to have a targeted therapeutic effect for diabetes (Guney et al., 2020; Qadir et al., 2020). Additionally, DYRK1A inhibitors have psychoactive properties and are often used as recreational drugs worldwide. Therefore, more research is needed before these agents can be used for beta-cell proliferation (Stewart, 2021).

Interestingly, some pancreatic cells, such as endocrine alpha cells, exocrine acinar cells, and ductal cells, have a certain amount of plasticity and can be transformed into insulin-producing phenotypes. Therefore, transdifferentiation of these cells to insulin-producing beta cells could potentially solve a high blood sugar problem. In addition, mouse acinar cells could be reprogrammed to have a beta-cell phenotype in vivo could by expressing three transcription factors (Pdx1, Ngn3, and MafA). Moreover, it was found that overexpression of a single transcription factor (Arx) can induce transdifferentiation of alpha cells to beta cells in mice (Honzawa & Fujimoto, 2021; Shapiro, 2018). This principle is currently being used to develop a gene therapy product, GPX-002, where alpha cells will be transformed into beta cells to produce insulin but will be distinct enough to evade the body’s immune system at the University of

Pittsburg in collaboration with the National Institutes of Health. GPX-002 is comprised of a novel infusion process that uses an adeno-associated viral (AAV) vector to deliver Pdx1 and MafA genes to the pancreas. These genes express proteins that transform alpha cells in the pancreas into functional beta-like cells to produce insulin but remain different from other beta cells to evade the body's immune system (National Insititutes of Health, 2021; Osipovich & Magnuson, 2018). The process is illustrated in Figure 2.12 (Osipovich & Magnuson, 2018).

Figure 2.12

Alpha to Beta Cell Programming



Note: Shows cell reprogramming. Adapted from "Alpha to beta-cell reprogramming: stepping toward a new treatment for diabetes" by Osipovich & Magnuson, 2018 (https://www.sciencedirect.com/science/article/pii/S1934590917305131) Copyright 2020 by Osipovich & Magnuson.

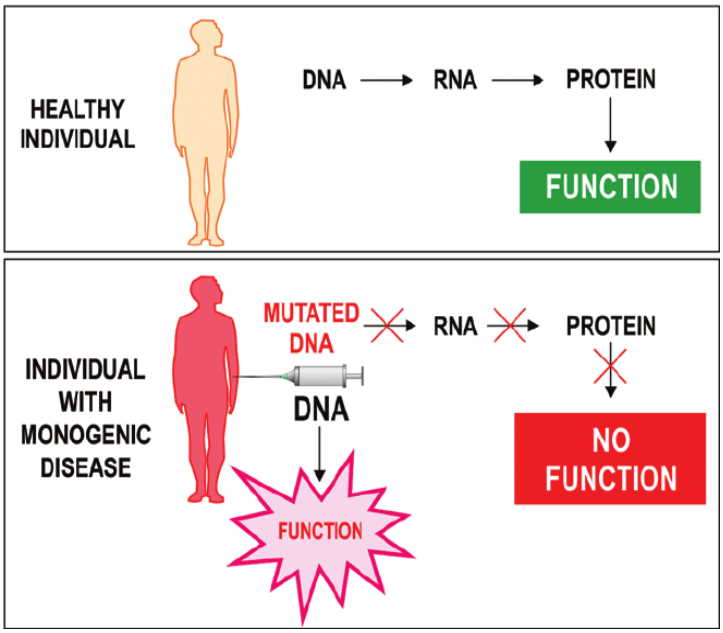
Another process is currently under ongoing research is "neogenesis" (new cell formation) to create new insulin-producing cells. In humans, new pancreatic beta cells are formed from ductal epithelium cells during embryonic development, stimulated by the transcription factor

Neurogenin3 (Ngn3). Intrestingly, it has been discovered that in some cases, the adult human body can form new pancreatic cells during a recovery process from a few pancreatic injury. Therefore, there is ongoing research on whether this process could be reactivated in the adult pancreas to generate new beta cells potentially. However, the method has not received much success so far (Guney et al., 2020).

Additional research is taking place in monogenic diabetes using gene-editing technology CRISPR/Cas9 in different forms of monogenic diabetes. Even though only 1-2% of the world population have monogenic diabetes, a lot of research is taking place in this area, hoping that if one gene could be corrected with gene therapy, several genes could be corrected in the future to cure polygenic diabetes. As discussed earlier, genetic information flows from DNA to RNA to a protein in a healthy individual. The process breaks down when a DNA is mutated and causes disease, as illustrated by Figure 2.13. With monogenic conditions, it is easier to pinpoint which gene should be corrected with gene sequencing (American Society of Gene and Cell Therapy, 2021b; Bellin & Dunn, 2020; Maxwell et al., 2020).

Figure 2.13

Gene Therapy for Monogenic Diabetes



Note: Adapted from "New tool in regenerative medicine: gene therapy" by Ruiz et al.,2012 (https://www.researchgate.net/figure/Schematic-illustration-of-gene-therapy-Insertion-of-genes-into-an-individuals-cell-and_fig1_223136850_. Copyright 2012 by Ruiz et al.

In 2018, Maxwell et al. successfully restored glucose homeostasis in diabetic mice with patient stem-cell-derived beta cells that were corrected for the WFS1 gene variant that caused Wolfram Syndrome. They used CRISPR-Cas9 to edit the gene (Maxwell et al., 2020). This success prompted a phase 1b/2a clinical trial of the therapeutic agent (called dantrolene sodium), to assess the safety, tolerability, and efficacy in pediatric and adult patients with Wolfram syndrome (Clinical Trial Number: NCT02829268i) (ClinicalTrials.gov, 2021a). The other type of monogenic diabetes is neonatal diabetes, where research is ongoing. Neonatal diabetes is caused by insulin gene mutations. Therefore, to solve this problem, Balboa et al. collected cells with insulin gene mutation from the patients, used CRISPR/Cas 9 to correct the gene in these cells, and then transplanted them into mice induced to have neonatal diabetes. The researchers

found that glucose homeostasis has been restored in those mice (Balboa et al., 2018). Barbetti et al. also reported similar findings with their studies (Ma et al., 2018).

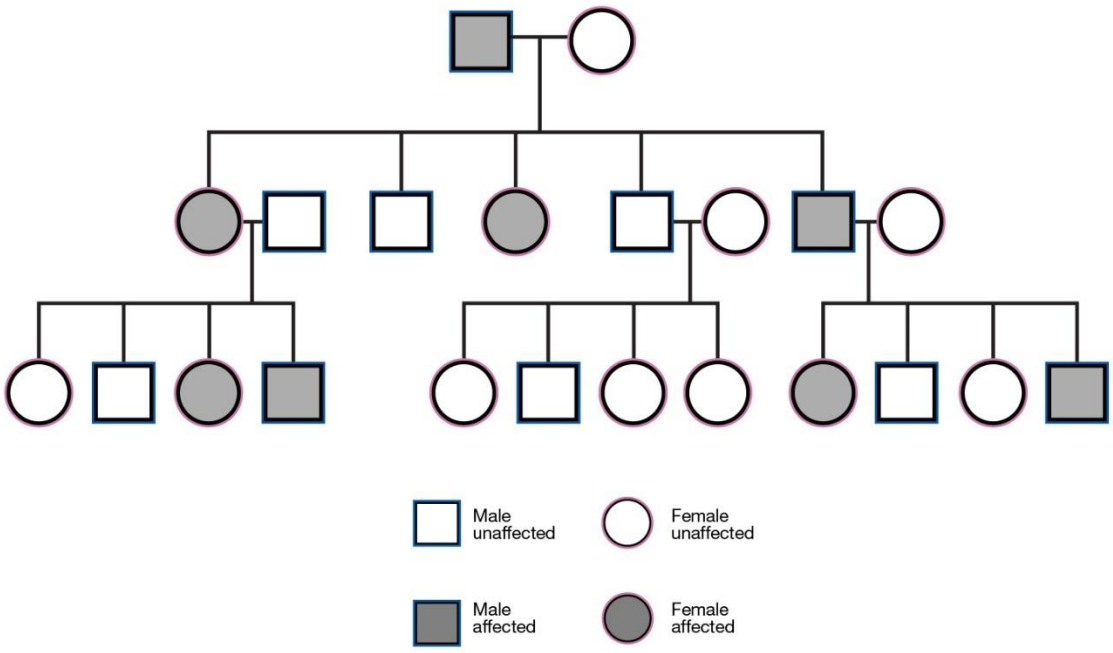
Barriers to Gene Therapy

The two main barriers to gene therapy are regulations and cost. Gene therapy provides exciting opportunities to cure many genetic disorders and cancers. However, just like any other emerging field, ethical boundaries need to be considered. Germline gene editing is prohibited in the United States, Europe, China, and many other countries due to the fear that it could be used for eugenics. In addition, as germline gene therapy takes place in the egg or sperm cells or early embryo, it comes with a very high risk of changing the overall genome of a person, possibly a dysfunctional one if not done correctly (American Society of Gene and Cell Therapy, 2020; National Academies of Sciences & Medicine, 2017).

On the other hand, with germline editing, certain autosomal dominant disorders and mitochondrial genetic disorders could be eliminated from the human species (American Society of Gene and Cell Therapy, 2020; National Academies of Sciences & Medicine, 2017). Autosomal dominance refers to a pattern of inheritance where only one copy of an inherited gene is sufficient to cause a disorder, in contrast to an autosomal recessive disorder where two copies are needed to inherit a disorder. Huntington’s disease and Marfan syndrome are typically autosomal dominant genetic disorders (National Human Genome Research Institute, n.d.). Children of an affected parent have a 50% chance of inheriting the condition, as illustrated by Figure 2.14 (National Human Genome Research Institute, n.d.).

Figure 2.14

Autosomal Dominant Disorder



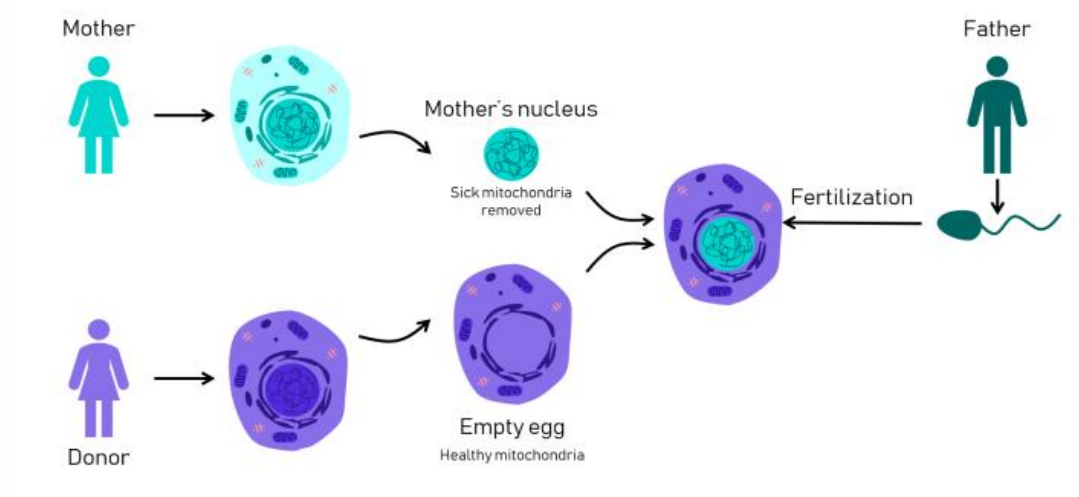
Note: Illustrates autosomal dominant disorders. Adapted from "Autosomal dominant" by National Human Genome Research Institute, n.d. (https://www.genome.gov/genetics-glossary/Autosomal-Dominant Copyright n.d, by National Human Genome Research Institute

Germline editing could also cure mitochondrial disorders. This disorder comes with a 100% chance of inheriting a disease from an affected mother as the mitochondrial genome is exclusively inherited from the mother. As previously discussed in this chapter, maternally inherited diabetes and deafness (MIDD), Wolfram Syndrome, and Fredrich's ataxia are such disorders. There are many other mitochondrial disorders in humans, such as Menkes disease, Gracile syndrome, and Leigh syndrome (Gorman et al., 2016). In 2016, Zhang et al. performed a germline gene therapy in Mexico and helped a couple with the birth of a healthy boy free of Leigh's syndrome. Typically, with this disease, a child's central nervous system is affected soon after birth, and the child dies within the first few years of age (J. Zhang et al., 2017). The

controversial technique Zhang et al. used, called having a “three-parent baby,” is now legal in the United Kingdom (National Academies of Sciences & Medicine, 2017). This method is illustrated in Figure 2.15. The process starts with a donor egg with healthy mitochondria. Then much of the genetic material is removed from the donor egg while leaving the healthy mitochondrial genes and the nutrients. At this stage, the mother’s genetic materials are inserted into the donor egg without the mitochondria. Then this egg is fertilized with the father’s sperm and implanted into the mother’s womb (Harvard University, 2021). The United States may need to relax gene therapy regulations in the future to allow this kind of germline therapy.

Figure 2.15

Germline Gene Therapy for Mitochondrial DNA Disorder



Note: Adapted from “Mitochondrial Transfer: the making of three-parent babies” by Harvard University, 2021(https://sitn.hms.harvard.edu/flash/2018/mitochondrial-transfer-making-three-parent-babies/).Copyright 2021 Harvard University

While somatic gene therapy is allowed in the United States and other countries, it still faces stricter regulations than other drug development processes. As discussed in this chapter, the tragic death of a gene therapy participant in the late 90s resulted in the United States’ moratoriums and suspensions on gene therapy research. In early 2000, the FDA lifted some of these moratoriums and sanctions, allowing gene therapy research to continue with strict

guidelines. In 2020, the FDA updated the guidelines on the follow-up timeline for a gene therapy treatment. The new guidelines suggest that studies using integrating vectors and genome-editing products follow patients for at least 15 years. For adeno-associated viral vectors, a minimum 5-year follow-up period is recommended (U.S. Food and Drug Administration, 2021a).

Additionally, gene-therapy trials are required to adhere to updated guidelines on good clinical practice specific to advanced therapy medicinal products. The European Union has similar approaches to the FDA. With these guidelines, it may take 15-20 years for a gene therapy product development and approval (U.S. Food and Drug Administration, 2021a; Nature Medicine, 2021).

High cost is another significant barrier to gene therapy. As illustrated by Table 2.3, one-time therapeutic use of Glybera costs $1 million, Imylygic costs $65,000, Luxturna costs $850,00, and Zolgensma costs $2.1 million (Cring & Sheffield, 2020).

Table 2.3

Cost of Certain Gene Therapy Products

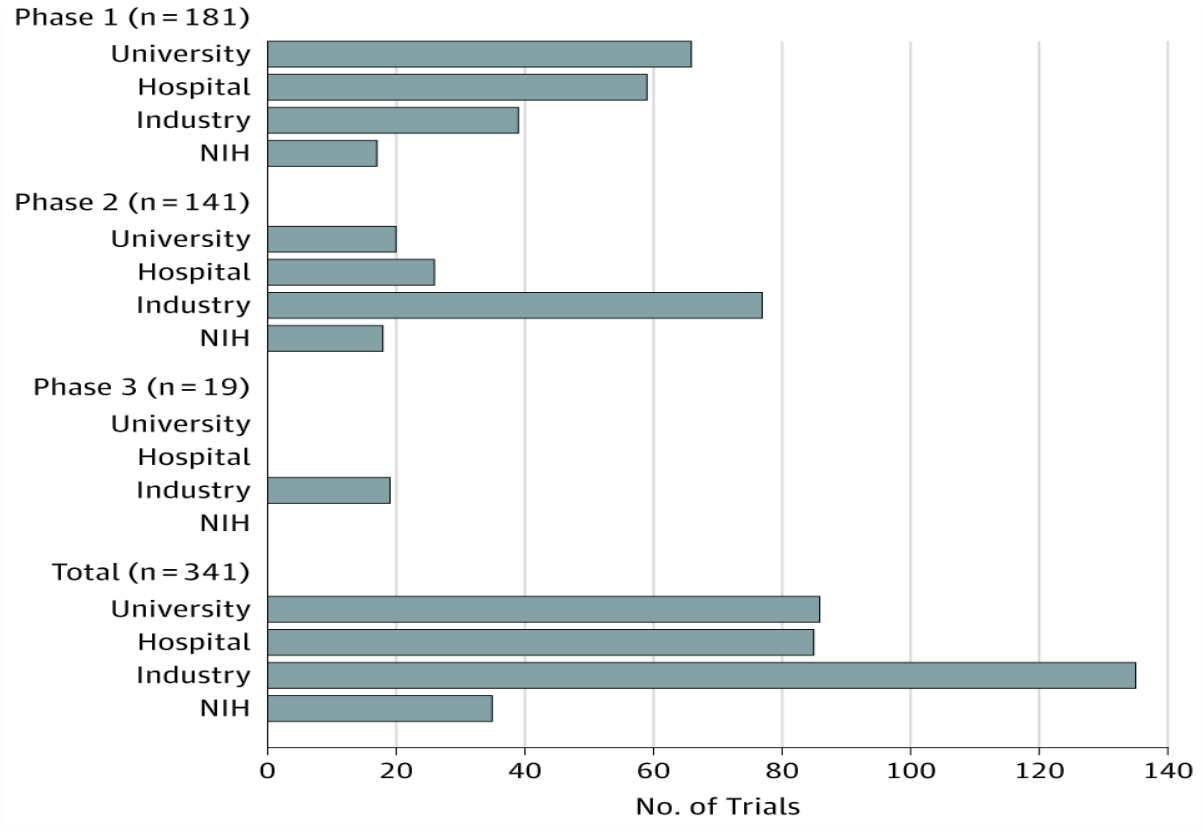
Drug	Year approved	Indication	Vector	Cost
Glybera (alipogene tiparvovec)	2012 (EMA)	"Adult patients diagnosed with familial lipoprotein lipase deficiency (LPLD) and suffering from severe or multiple pancreatitis attacks despite dietary fat restrictions"	AAV	$1 million
IMLYGIC (talimogene laherparepvec)	2015 (FDA/EMA)	"Unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after initial surgery"	Herpes Simplex Virus	$65,000
Luxturna (voretigene neparvovec-rzyl)	2017 (FDA) 2018 (EMA)	"Biallelic RPE65 mutation-associated retinal dystrophy"	AAV	$850,000
ZOLGENSMA (onasemnogene abeparvovec-xioi)	2019 (FDA)	"Pediatric patients less than 2 years of age with spinal muscular atrophy (SMA) with bi-allelic mutations in the survival motor neuron 1 (SMN1) gene"	AAV	$2.1 million

Note: Adapted from ‘Current FDA and EMA-approved gene therapies” by Cring & Sheffield, 2020 (https://www-nature-com.radford.idm.oclc.org/articles/s41434-020-00197-8#Tab1) Copyright 2020 by Cring and Sheffield.

This is reflected by the estimated cost of research and development of a new drug that ranges from $161 million to $2 billion, including all phases of clinical trial costs and 15–20-year follow-up. Kassir et al. investigated the funding of all active gene therapy trials until January 2019. They found that mainly private industries have been carrying the cost of this kind of drug development. Figure 2.17 illustrates this (Kassir et al., 2020). More government funding and relaxation of certain regulations will encourage competition in gene therapy product development and bring down the overall cost for the patients (Kassir et al., 2020).

Figure 2.16

Funding for Gene Therapy Clinical Trials



Note: Adapted from “Sponsorship and funding for gene therapy trials” by Kassir et al., 2020. (https://jamanetwork.com/journals/jama/fullarticle/2762298) Copyright 2020 by Kassir et al.

Previous Systematic Reviews and the Gap in the Literature

To find previous systematic reviews for diabetes treatments with controlled trials, PubMed via Medline, Embase, Web of Science, EBSCO, CINAHL, ScienceDirect, and Cochrane Database of Systematic Reviews were searched. The search strategy limited the time frame from 2000 to 2021, language preference to English, and subjects to type 1 diabetes, type 2 diabetes, all diabetes, randomized controlled and clinical trials whenever applicable. The search resulted in 13 systematic reviews in diabetes treatments that included only one systematic review in gene therapy for diabetes. The Cochrane Library and the International Prospective Register of Systematic Reviews (PROSPERO) were also searched for systematic reviews protocols in case there might be ongoing systematic reviews in gene therapy for diabetes that did not show up in the other databases. However, no protocol in gene therapy for diabetes has been found in either of these databases that house registered protocols for ongoing systematic reviews.

The 13 systematic reviews that were found centered on various types of controlled or randomized controlled interventional studies for diabetes. Three systematic reviews (Cao et al., 2021; Giugliano et al., 2011; Kalafat et al., 2018) investigated the efficacy of different pharmaceutical agents in people with diabetes; specifically, how metformin can prevent hypertensive disorders of pregnancy in women with gestational diabetes or obesity, how sodium-glucose cotransporter-2 inhibitors can benefit kidney and cardiovascular outcomes for patients with type 2 diabetes mellitus and chronic kidney disease and treatment regimens, and how insulin analogs can help lower hemoglobin A1c to less than 7% in people with type 2 diabetes. Another systematic review focused on regulating hyperglycemia after stroke (Laird & Coates, 2013).

Four of the systematic reviews investigated approaches to treatment. Engebretson and Kocher (2013) were interested in periodontal treatment and found that it improves diabetes outcomes in patients. Moussa et al. (2018) investigated vitamin D supplementation for improvement of chronic low-grade inflammation in patients with type 2 diabetes. Costello et al. (2016) and Suksomboon et al. (2011) investigated chromium and vitamin E supplements for glycemic control in people with type 2 diabetes.

Other reviews investigated alternative treatments. Hochsmann and colleagues (2016) examined the effect of active video games in overweight individuals with diabetes, whereas Patil and team (2018) considered the impact of peer support interventions on cardiovascular disease risk factors in adults with diabetes. Yu et al. (2018) explored the effectiveness of traditional Chinese medicine-based lifestyle interventions on biomedical, psychosocial, and behavioral outcomes in individuals with type 2 diabetes. Researchers were also interested in addressing systematic issues impacting care. Terens et al.'s (2018) review focused on quality improvement strategies at the primary care level to reduce inequalities in diabetes care for patients.

The only systematic review that was found on gene therapy examined gene therapy studies in rodents that were induced to have type 1 diabetes mellitus (Ghiasi et al., 2020). In this review, entitled, "Efficacy of insulin targeted gene therapy for type 1 diabetes mellitus: A systematic review and meta-analysis of rodent studies," Ghiasi et al. evaluated 16 studies: 12 of which used viral vectors, and four of which used non-viral vectors. The authors found that gene therapy through both methods effectively reduced blood glucose and increased insulin production for at least 500 days without any adverse effects. The researchers also conducted a meta-analysis and found that gene therapy with viral vectors decreased mean intraperitoneal glucose tolerance test (IPGTT) by 12.69 mmol/l, fasting blood glucose by 13.51 mmol/l, and

insulin production by 398.28 pmol/l. In contrast, gene therapy with non-viral vectors reduced fasting glucose by 29.95 mmol/l and insulin production by 114.92 pmol/l. Overall, the authors found that gene therapy with viral vectors was more effective than gene therapy with non-viral vectors in rodents (Ghiasi et al., 2020).

Out of the 13 systematic reviews discussed here, 12 studies examined a wide range of interventions for diabetes in humans: pharmaceutical agents, alternative medicine, supplementations, gum disease treatment, peer support, equity, active video gaming, and blood sugar management after stroke (Cao et al., 2021; Costello et al., 2016; Engebretson & Kocher, 2013; Giugliano et al., 2011; Höchsmann et al., 2016; Kalafat et al., 2018; Laird & Coates, 2013; Mousa et al., 2018; Patil et al., 2018; Suksomboon et al., 2011; Terens et al., 2018; Yu et al., 2018), but no interventions with gene therapy. Even though an extensive search was conducted through nine databases, the search resulted in only one systematic review in gene therapy for diabetes that was conducted on rodent studies (Ghiasi et al., 2020). It is evident that there is a significant gap in the literature on gene therapy for diabetes in humans. Therefore, this systematic review will address this particular gap in knowledge and focus on studies in gene therapy for diabetes in humans.

Summary

Diabetes is a physically and financially debilitating genetic disorder. It is one of the top 10 causes of death, one of the leading six causes of disability, and the fastest-growing health problem globally (International Diabetes Federation, 2020). According to the International Diabetes Federation (2019a), there will be 578 million adults with diabetes by 2030 and 700 million by 2045 (International Diabetes Federation, 2019a). Many improvements have taken place in diabetes treatment from the early 1920s to 2015 (White, 2014). However, these

treatments only alleviate symptoms and delay disease progression but do not cure diabetes. From 2015 onwards, a better understanding of the pathogenesis of diabetes at a cellular and genetic level led to research for a cure for diabetes (Kahn et al., 2015). All forms of diabetes have genetic links, either polygenic or monogenic. Moreover, the manifestation of diabetes occurs due to a decline in insulin production. Therefore, genetic modification to intensify insulin production would be the most effective way to cure all types of diabetes.

Regrettably, due to strict regulations, 15-20 years of dedication required for different phases of clinical trials, and the lack of government funding for research and development, very few gene therapy products are available to people (Kassir et al., 2020). So far, only 22 gene therapy products have been approved for human use worldwide (U.S. Food and Drug Administration, 2021a). Most of these products are for the treatment of cancer and cardiovascular diseases, but none of them are for the treatment of diabetes (Nóbrega et al., 2020). It is clear from this data that not much research has occurred in gene therapy for diabetes earlier in this century. Therefore, it should not be surprising that no systematic review on gene therapy for diabetes in humans has been found thus far because it is likely that the researchers did not have enough studies on this topic to pull from to conduct a systematic review.

However, research in gene therapy for diabetes in humans picked up its pace in recent years. Gene therapy with glucagon-like peptide-1 (GLP-1) has successfully increased gene expression, stimulated insulin secretion, and aided in beta-cell survival in patients with type 2 diabetes (National Institute of Diabetes and Digestive and Kidney Diseases, 2016; U.S. Food and Drug Administration, 2019a). Current research is taking place in how to genetically reengineer porcine beta cells into functional human beta cells to produce insulin (Bellin & Dunn, 2020), how to genetically convert pancreatic alpha cells into functioning beta cells that would not

require immunosuppressant drugs (Osipovich & Magnuson, 2018), and how to genetically stimulate beta cells to proliferate, regenerate, and transdifferentiate (Guney et al., 2020; Qadir et al., 2020). These studies are now in preclinical or clinical stages (Bellin & Dunn, 2020; Guney et al., 2020; Osipovich & Magnuson, 2018). Unfortunately, no systematic review has been found to have conducted on this research to help patients with diabetes decide if gene therapy might be a better option for them compared to current pharmaceutical agents. So far, only one systematic review has been found that focused on gene therapy for type 1 diabetes in rodents. Therefore, this systematic review will address this gap in the literature and determine if gene therapy for diabetes is more effective than pharmaceutical agents in humans.

Chapter Three

Methodology

Diabetes is a lifelong disease with expensive care, lower quality of life, and increased morbidity and mortality (International Diabetes Federation, 2019b). For diseases such as diabetes, evidence-based practice of medicine can provide the most effective care and improve patient outcomes. Evidence-based medicine requires understanding and interpreting the scientific evidence and applying it to clinical practice, where systematic reviews of clinical trials are at the top of the levels-of-evidence pyramid (Linares-Espinós et al., 2018; Livinski, 2015; Pollock & Berge, 2018). Therefore, a systematic review of controlled clinical trials of gene therapy for diabetes was conducted for this project.

As the name implies, a systematic review utilizes systematic and reproductive methods to identify, select, and critically appraise all relevant research to a focused review question. Therefore, the systematic search should be comprehensive, organized, transparent, and reproducible so that the conclusions are as unbiased and closer to the truth as possible and conducive to evidence-based medicine. To achieve these, systematic reviews usually follow eight consecutive steps: 1) formulate the review question, 2) define inclusion and exclusion criteria, 3) develop a search strategy and locate studies, 4) select studies, 5) assess study quality, 6) extract data, 7) synthesize and analyze data, and 8) report findings (Linares-Espinós et al., 2018; Pollock & Berge, 2018). For this systematic review, these steps were followed. The details of these steps are described below.

Formulating the Review Questions

Developing a well-formulated review question is essential for a systematic review to justify carrying out the study and guide critical parts in the review process, such as selecting the studies, search strategies, and data extraction. The widely used model is an acronym, PICO, that

identifies components of clinical evidence such as the population (P) under study, the intervention (I) or treatment being evaluated, the comparison (C) of that intervention, and the outcomes (O) (Linares-Espinós et al., 2018; Pollock & Berge, 2018). Consequently, *The Cochrane Handbook for Systematic Reviews of Interventions* specifies using PICO as a model for developing a review question to ensure that the relevant components of the question are well-defined (Higgins et al., 2019).

There are many benefits to using PICO. For instance, the PICO format forces the researcher to focus on the most critical concern and the favorable outcome for a patient with a particular disease. Additionally, it directs the researcher to clearly identify the problem, intervention, and outcomes related to specific care provided to a patient. Moreover, it helps the researcher develop effective search strategies by prompting the researcher to select key terms and their synonyms for each component of the model, such as the population (P), the intervention (I), the comparison (C), and the outcome (O) and facilitates the search process (Eriksen & Frandsen, 2018). Therefore, for this systematic review, a PICO model was used to formulate the review questions. As the purpose of this systematic review is to determine whether gene therapy was an effective diabetes treatment and diabetes related complications, the population in this regard is patients with diabetes (P), intervention is gene therapy (I), comparator (C) is pharmaceutical agents or placebo, and the outcome (O) is the management of diabetes and its related complications. Using these four elements, the following review questions were formulated.

RQ1: In patients with type 1 diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes (O)?

RQ2: In patients with type 2 diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes (O)?

RQ3: In patients with diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes-related neural/nerve disorders (O)?

RQ4: In patients with diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing critical limb ischemia (O)?

RQ5: In patients with diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes-related eye complications (O)?

Defining the Inclusion and Exclusion Criteria

The PICO model utilizes the key concepts for the review, thereby aiding the development of the inclusion and exclusion criteria for the review. Defining characteristics of these key concepts also set the scope of the systematic review described in Table 3.1. In the PICO model, the first element is population (P), and the inclusion criterion was patients with any type of diabetes, and the exclusion criterion was patients without diabetes. The population included all ages, races, and sexes. The second element was intervention (I), and the inclusion criterion for was any gene therapy for diabetes and diabetes related complications, and the exclusion criterion was other treatments for these conditions. The third element was comparison (C). The inclusion criterion for this element was pharmaceutical agents for diabetes or placebo, and the exclusion criteria was bariatric/weight loss surgery, herbal treatments, and nutritional supplements. The outcome (O) was the fourth element of PICO. The inclusion criteria for the outcome were normal blood sugar level, normal HbA1c level, insulin consumption, c-peptide level, and other measurements that would evaluate improvements with diabetes related complications.

As the focused review questions specified gene therapy as an intervention and pharmaceutical agents or placebos as controls, only interventional studies with controls (controlled trials) were suitable for this systematic review. Therefore, this systematic review search included all interventional controlled trials from publications and excluded other study designs. The time frame for the review was from 2000 to 2022, and it excluded any year before 2000. This ensured inclusion of as many interventional studies as possible over the last two decades. Furthermore, this review included any sample population sizes and ages to ensure all forms of diabetes and related complications are included and excluded no sample sizes or ages. All included publication were in the English language, and the review excluded any other language.

Table 3.1

Inclusion and Exclusion Criteria

	Inclusion	Exclusion
Population (P)	Patients with any type of diabetes All ages, races, and sexes	Patients without diabetes
Intervention (I)	Gene therapy for diabetes and diabetes related complications	Other treatments for diabetes
Comparison (C)	Pharmaceutical agents for diabetes or placebo	Bariatric/weight loss surgery, herbal treatments, nutritional supplements
Outcome (O)	Normal blood sugar level, normal HbA1c level, insulin consumption and c-peptide level, measurements for diabetes related complications	No exclusions
Study Design	All controlled trials from publications including grey literature	Other study designs
Publication Language	English	Any other language
Time Frame	2000-2022	Before 2000
Sample Population Size	Any	None

Developing a Search Strategy and Locating Studies

Designing successful search strategies requires knowledge of different databases, indexing, and database structures. Therefore, it is important to consult experienced information specialists to build a successful search strategy. In fact, in 2011, the Institute of Medicine specified one of the standards for satisfactory systematic reviews is to work with a librarian or other information specialist trained in performing systematic reviews to plan a search strategy and conduct searches (Eden et al., 2011; National Institutes of Health Library, 2022). Additionally, there has been increasing evidence that supports that involving an information specialist in a systematic review process improves the quality of the search process (Meert et al., 2016; Metzendorf, 2016; Rethlefsen et al., 2015). Therefore, the researcher worked with the Head Librarian at the Radford University Carilion Library and verified the search process.

An iterative process was used to develop the most successful search strategy. One of the advantages of developing a focused review question is that the PICO components can be used to develop appropriate search strategies. Thus, the initial search strategy was developed based on the PICO components, possible synonyms, and Boolean operators. An example of that search strategy is shown in Table 3.2. This initial search strategy was tried in the pre-selected databases to assess the efficacy of the search strategy for identifying relevant articles. Unfortunately, this strategy was found to be too narrow in all ten preselected databases that yielded no results. Therefore, a general search string “diabetes” AND “gene therapy” was used in each database and necessary refinement were made based on the initial results. The refinement processes were documented to prevent a repetition of search approaches that have previously been tried. The final search strategy was recorded in detail and described in Appendix A to demonstrate transparency and reproducibility of the process.

Table 3.2

Search Strategy Based on PICO & Boolean Operators

Population/ Problem		Intervention		Comparison		Outcome
Patients with high blood sugar OR Hyperglycemia OR diabetes OR diabetes mellitus OR type 1 diabetes OR type 2 diabetes OR monogenic diabetes OR polygenic diabetes	AND	Gene therapy randomized controlled trials OR randomized controlled preclinical trials OR randomized controlled clinical trials	AND	Insulin therapy OR Meglitinides OR Nateglinide OR Repaglinide OR Sulfonylureas OR Glipizide OR Glucotrol OR Glimepiride,etc	AND	Normal blood sugar OR normoglycemia OR Euglycemia OR normal HbA1c level OR normal insulin level OR normal c-peptide level

Database Selection for Journal Articles

A comprehensive systematic review aims to find as many potentially relevant studies as possible to minimize bias. Since the topic for this systematic review is a biomedical one, the principal biomedical databases were the first line of inquiry for relevant studies. Medline (via Ovid or PubMed), Embase, Scopus, and Web of Science are the primary biomedical databases (National Institutes of Health Library, 2022). The Cochrane collaboration recommends using Medline, Embase, and the Cochrane Central Register of Controlled Trials (CENTRAL) at a minimum for adequacy (Higgins et al., 2019). Additionally, recent studies found that using Medline, Embase, Web of Science, and Google Scholar at a minimum is necessary for adequate

and efficient coverage of published literature for systematic reviews (Bramer et al., 2017; Gusenbauer & Haddaway, 2020).

However, the authors warn that Google Scholar searches are not as transparent and reproducible as systematic reviews require since the search algorithm uses texts instead of a Boolean algorithm. Moreover, Google Scholar is updated frequently; therefore, using the same texts tends to produce different results for different users at other times. Furthermore, Google Scholar lacks filters to select specific types of studies, thereby returning a large number of results with various kinds of reports, making it difficult to find all relevant papers suitable for a systematic review (Bramer et al., 2017; Haddaway et al., 2021; Piasecki et al., 2018).

Therefore, for this systematic review, Google Scholar was not used. Instead, Medline, Embase, Web of Science, Scopus, and CENTRAL were used to find relevant studies. Medline is the United States National Library of Medicine's primary citation database indexes over 5,400 journals and 22 million citations. Embase is Elsevier's biomedical and pharmacological bibliographic database that provides current biomedical and drug literature and the most frequently used databases for drug information by reviewers. Web of Science is Thomson Reuter's science database indexes over 12,000 journals. Scopus is Elsevier's database that provides journal articles, books, book chapters, and conference proceedings in biological sciences such as genetics and molecular biology. CENTRAL is considered the most comprehensive source of reports of randomized controlled trials as it contains over 1,275,000 trials/trial registry records (Higgins et al., 2019; Masic & Milinovic, 2012; National Institutes of Health Library, 2022).

Moreover, Medline, Embase, Web of Science, and CENTRAL use Boolean algorithms and offer filters to narrow down specific types of studies (Higgins et al., 2019; National Institutes

of Health Library, 2022). As this systematic review only included controlled trials, these filters helped choose this type of study. Medline via PubMed, Embase, Web of Science were accessed through Radford University Carilion Library, Scopus through the National Library of Medicine, and CENTRAL through the Cochrane Library.

Sources for Grey Literature

In addition to the databases, controlled trials included in the grey literature were located for this systematic review. Grey literature refers to information outside the mainstream of published journals not controlled by commercial publishers, such as reports or dissertations, conference papers, governmental or private sector research, and clinical trials that are either ongoing or unpublished (National Institutes of Health Library, 2022). Research shows that including grey literature is essential in conducting systematic reviews to reduce publication bias since many scientifically valid studies, especially those with negative results, are never published (Haddaway et al., 2015; Paez, 2017). The Cochrane Collaboration and National Institutes of Health recommend using grey literature for a comprehensive systematic review to overcome publication bias, especially for studies investigating the effectiveness of therapeutic interventions since published literature tends to highlight only the positive effects of interventions (Higgins et al., 2019; National Institutes of Health Library, 2022). Since this systematic review will examine the effectiveness of gene therapy for diabetes, it was imperative to consider grey literature to reduce publication bias.

The main form of grey literature that is used to answer a therapy question is clinical trials, especially ongoing trials, and trials with negative results. These are mainly available through trial registries and results databases. The clinical trials.gov, available at www.clinicaltrials.gov, is a U.S. government resource that provides trial information and

sometimes results and outcomes of publicly and privately supported clinical studies conducted in the United States and other countries. However, trial registration is voluntary and not required by law; therefore, not all clinical trials information might be available through this site. Information on additional clinical trials could be found at https://trialsearch.who.int/. It is an international clinical trials registry site that the World Health Organization maintains (National Institutes of Health Library, 2022). Moreover, pharmaceutical companies are the primary producers of therapeutic drugs and publish information about ongoing clinical trials in their own trials databases such as the Clinical Trials Portal of the International Federation of Pharmaceutical Manufacturers and Associations available at https://www.researchinformation.info/search/node/. For this systematic review, all three-trial registry were searched for relevant studies. Additionally, Grey Literature Network at http://www.greynet.org/opengreyrepository.html and U.S. Federal Science Alliance at https://www.science.gov/scigov/desktop/en/search.html provide information on studies taking place at universities or governmental institutions that were not registered with any trial registries (National Institutes of Health Library, 2022; Tufts University Library, 2022). Therefore, these two sources were also searched to locate additional controlled trials. To summarize, a total of 10 sources, five databases to find peer-reviewed journal articles, and five sources to find grey literature were used for this systematic review, listed in Table 3.3.

Table 3.3

Sources to Be Used

Databases for peer-reviewed journal articles	Sources for grey literature
1.PubMed	1. Clinicaltrials.gov
2. Embase	2. WHO clinical trials site
3. Web of Science	3. Clinical Trials Portal of the International Federation of Pharmaceutical Manufacturers and Associations

4. Scopus	4. Grey Literature Network Service (GreyNet)
5. CENTRAL	5. US Federal Science Alliance

Selecting Studies

Generally, a study selection process for a systematic review includes nine steps: 1) merging reference managers with databases to ensure that reports can be imported, 2) running searches on the databases, 3) examining search results, reviewing titles and abstracts and removing irrelevant reports, 4) importing all relevant reports, 5) reviewing imported full-text reports and ensuring compliance with predetermined inclusion criteria, 6) making final decisions on study selection, 7) removing duplicate journal reports, and 8) linking separate reports of the same study, and 9) recording ongoing trials that have not yet been reported in the bibliographic databases (Higgins et al., 2019; Linares-Espinós et al., 2018; Livinski, 2015). For this systematic review, these steps were followed for study selection.

In addition, methodological experts recommend that two reviewers should independently determine the eligibility of studies for inclusion in a systematic review, a process referred to as the “interrater reliability” where a “kappa calculation” is performed to determine the level of reliability (Linares-Espinós et al., 2018; Livinski, 2015). Therefore, the researcher and an external interrater independently rated each imported study for inclusion or exclusion based on a coding protocol that was developed using the predetermined inclusion criteria. Then, a kappa calculation on the ratings were performed to determine the inter-rater reliability of the selection process, displayed in Appendix B. The kappa coefficient was 0.93. For kappa results, values ≤ 0 indicate no agreement and 0.01–0.20 mean none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement (McHugh, 2012). Therefore, the interrater agreement for this systematic review, 0.93, indicates that the level of agreement was almost perfect.

Assessment of Study Quality

Systematic reviews depend solely on evidence gathered from other studies to draw conclusions about interventions. Therefore, those conclusions could be just as biased or unbiased as the included studies. Consequently, it is crucial to assess the methodological quality of each study to reduce bias. National Center for Biotechnology Information recommends a Jadad scale to assess the methodological quality of controlled trials. With this 7-item scale, studies are scored according to key features of a superior quality clinical trial such as randomization, masking, and patient adherence. The final score ranges from 0 to 5 points with higher scores indicating better quality studies (Berger & Alperson, 2009; The National Center for Biotechnology Information, 2017). This scale was used to the assess level of bias and the quality of included studies in this systematic review. Out of the 47 included studies, 21 studies rated at 5, four studies rated at 4, four studies rated at 3, 16 rated at 1, and two rated at 0. The median Jadad score was 4 with an interquartile range of 4 indicating that the studies included in this systematic review were of high quality and had a low risk of bias. The items for calculating a Jadad score and guidelines for assessment are included in Appendix B. The individual Jadad scores for the studies are included in the data extraction table included in Appendix C.

Extracting Data

Data was extracted based on the PICO components as they answered the review question the best. Additionally, information about study design, trial numbers, author names, sponsorship, date, and types of diabetes were recorded to avoid duplication of data. Extracted data was recorded in the proposed data extraction form. Extracted data was recorded in the proposed data extraction form provided in Table 4, displayed in Appendix D.

Table 3.4

Data Extraction Form

Study Design & Trial Numbers	Authors & Sponsors if any	Date and Duration	Type of Diabetes	Population (P) Age, Sex, Race, Number of participants	Intervention (I) Gene Therapy	Comparison (C) Pharmaceutical Agents	Outcome (O) Normal blood sugar level, Normal HbA1c level, insulin consumption and C-peptide level

Data Synthesis and Analysis

Since this systematic review is not including a meta-analysis, a narrative synthesis of the included studies was provided. A narrative synthesis uses a textual approach to analyze the relationships within and between studies and gives an overall assessment of the robustness of the evidence for the effectiveness of a specific intervention. The Cochrane collaboration recommends a 4-step approach to a narrative synthesis of systematic review: 1) identification of key theories/pathways of how the intervention works, why it works, and for whom it works, 2) developing a preliminary synthesis of the findings of included studies, 3) exploring the relationship in the data within and between studies, and 4) assessing the robustness of the synthesis (Higgins et al., 2019). First, the pathways to gene therapy for diabetes, how it works, and the study population were identified in the studies. Then a preliminary synthesis of the findings of the included studies were developed, using the PICO characteristics. Then, these characteristics were compared among the studies and the studies were grouped together based on similar characteristics. After that, a narrative synthesis of each study was provided in each group.

The quality of the evidence was calculated through Jadad scale and the median score of the studies found to be 4, indicating that the studies were of good quality and the results had low bias. Finally, the results of the studies were used to answer the review questions.

Reporting Findings

Since this systematic review includes studies from bibliographic databases, trial registries, and other sources, the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram that includes searches of databases, registers, and other sources was the most suitable format for reporting (Haddaway et al., 2021). A completed diagram detailing the process of identification, screening, and inclusion of reports was produced using the Shiny App from the PRISMA, displayed in Appendix E.

Chapter Four

Results

Using an initial search strategy and predetermined inclusion criteria, records were identified from 10 preselected sources: PubMed (n = 21), Embase (n = 138), Web of Science (n = 16890), Scopus (n = 97167), CENTRAL (n = 51), Grey Literature Network Service (n = 1), U.S. Federal Science Alliance (n = 285), Federation of Manufacturers Associations (n = 0), Clinicaltrials.gov (n = 113), WHO Trials site (n = 3). The search produced a total of 114,669 records. As the number of records was too high to scan all the abstracts, and many records seemed unrelated to the topic, an iterative search strategy was used at this point to find more specific records. The process varied per database, offering different filters to narrow the search process. Using the filters successfully removed 114, 081 records. The final search strategy per database was recorded and displayed in Appendix A for transparency and reproducibility of the process. Additionally, the researcher removed 289 duplicate and unrelated records from the total number of reports. The researcher then screened the remaining 299 abstracts, and 170 unrelated records were removed. After that, the remaining 129 articles were imported to the researcher's reference manager EndNote. However, 76 of these articles were duplicates and therefore removed using EndNote's "deduplication" feature. Four other articles were removed from the final analysis as three of the studies did not include people with diabetes in their final recruitment, and the fourth one had nutritional supplements (predefined as the exclusion criteria for this systematic review) as one of their interventions. Finally, 47 trials were deemed suitable for the systematic review. Some trials did not have any results updated on the clinical trials sites. Therefore, additional Google searches were conducted, and three press releases with interim results were found on the sponsor sites. These press releases were imported to EndNote and included in the final narrative synthesis of the studies. The step-by-step identification, screening,

and inclusion process are shown using the PRISMA Flow Diagram in Appendix E. The narrative synthesis of the studies, review questions, and outcomes are provided in this chapter.

Narrative Synthesis of the Studies

Type 1 Diabetes

Twenty-four studies were found on type 1 diabetes, an autoimmune disorder where the body's immune system destroys its own cells, particularly pancreatic beta cells giving rise to insulin deficiency (UCSF, 2022). Therefore, the researchers are conducting trials targeting different parts of the malfunctioning immune system with gene therapy for type 1 diabetes. Studies with similar gene therapy pathways were grouped and synthesized, narrated below.

Interleukin Studies. Interleukin-2 (IL-2) is an important player in immune regulation or tolerance of the body's own cells. This cytokine (cell signaling) molecule induces T regulatory (Treg) cells' ability to suppress the destruction of insulin-producing beta cells, thereby potentially increasing Treg function to halt disease progression in type 1 diabetes (Vaillant & Qurie, 2021). Because of this, many researchers are investigating IL-2 gene therapy or Treg cell gene therapy products for type 1 diabetes. Six studies focused on IL-2 gene therapy to either halt progression or cure type 1 diabetes (Hartemann et al., 2013; Iltoo Pharma, 2015; Marcovecchio et al., 2020; Rosenzwajg et al., 2020; Seelig et al., 2018; Todd et al., 2016a).

In 2011, Hertemann et al. launched a single-center, phase I/II clinical trial (NCT01353833) to investigate the effect of low-dose interleukin-2 in 24 patients, 18-55 years old, with established insulin-dependent type 1 diabetes and at least one diabetes-related autoantibody. The patients were randomly assigned to four cohorts (in a 1:1:1:1 ratio) to receive either placebo or IL2 at 0.33 unit/day, 1 unit/day, or 3 units/day for a 5-day course and followed for 60 days. There were no serious adverse events. Change in Treg cells was measured by flow

cytometry. IL2 induced a dose-dependent increase in the proportion of Treg cells, 2.8% increase in the 0.33 unit/day group, 3.9% increase in the 1 unit/day group, and 4.8% in the 3 units/day group. Additionally, the glucose-metabolism variables, such as the daily blood sugar level, C-peptide level, and HbA1c, were found to remain stable over the IL-2 trial period. While further investigation is warranted, this study sheds light on IL-2's potential to stop the progression of type 1 diabetes (Hartemann et al., 2013).

Another IL-2 product, Aldesleukin, was investigated by Todd et al. (2016b). Aldesleukin is a human recombinant IL-2 product produced by recombinant DNA technology using a genetically engineered E. coli strain that expresses an analog of the human IL-2 gene. The investigation was conducted via two clinical trials, where each trial had two phases. The first trial (NCT01827735) was a single-center non-randomized, single dose, open-label study with 40 patients from 2013 to 2015. The trial's objective was to identify the best doses of IL-2 to induce targeted increases in Tregs. There was an initial learning phase with five pairs of participants, each receiving one of five preassigned single doses (from 0.04 × 106 to 1.5 × 106 IU/m2), to model a dose-response curve. The results obtained from each participant were then incorporated into interim statistical modeling to target the two doses most likely to induce 10% and 20% increases in Treg frequencies. The primary patient population analysis revealed that the optimal doses of Aldesleukin to induce 10% and 20% increases in Tregs were 0.101 × 106 IU/m2 and 0.497 × 106 IU/m2, respectively. However, the effect of a single dose of Aldesleukin tapered off within 2-3 days requiring repetition (Todd et al., 2016b).

Therefore, the researchers designed the subsequent trial to determine a repeat dosing establishing a steady-state of Treg frequency increase by 20%-50%, with the eventual goal of preventing Type 1 diabetes (Todd et al., 2016b). This trial (NCT02265809) was a non-

randomized, open-label, response-adaptive study with 38 participants aged 18-70 with type 1 diabetes. The initial learning phase allocated 12 participants to six different predefined regimens. Then, three cohorts of eight participants were sequentially given dose frequencies based on repeated interim analyses of all accumulated trial data. Thirty-six participants completed the treatment. The authors found that the optimal regimen to maintain a 30% steady-state Treg increase is 0.26 × 106 IU/m2 every three days (Seelig et al., 2018).

Yet another dose-finding study (NCT01862120) of interleukin-2 was carried out from 2016 to 2017 (Rosenzwajg et al., 2020). This trial included 24 children, 7 to 14 years old, with recently diagnosed diabetes. This study was a multicenter, double-blinded, placebo-controlled, dose-finding phase I/II clinical trial conducted in four centers (7/5/6/6 patient distribution) at university hospitals in France. The subjects received placebo or IL-2 at doses of 0.125, 0.250, and 0.500 million international units (MIU), respectively, given daily for a 5-day course and then fortnightly for one year. The researchers found that IL2 induced a dose-dependent increase in the mean proportion of Tregs, from 23.9% at the lowest to 77.2% at the highest dose, significantly different from placebo for all dose groups. While the individual Treg responses to IL-2 were variable and fluctuated over time, seven patients, all among those treated with the 0.250 and 0.500 MIU m-2 day-1 doses, were Treg high responders. No significant change was detected in glycemic control in any dose group compared with placebo. However, there was improved maintenance of induced C-peptide production at one year in the seven Treg high responders compared with low responders. This result indicates that the treatment possibly halted the beta cell destruction in these patients (Rosenzwajg et al., 2020).

Two trials are currently ongoing. Another clinical trial (NCT03782636, EudraCT 2017-002126-20) on Aldesleukin was launched in 2019 by the University of Oxford and is currently in

phase II of the study (Marcovecchio et al., 2020). This study is a multicenter, double-blind, randomized, placebo-controlled trial in 45, 6 to 18 years old patients within six weeks of type 1 diabetes diagnosis. The subjects were randomized in a 2:1 ratio to receive either Aldesleukin (0.2 x 106 IU/m 2 twice weekly) or a placebo for 6 months. The primary objective of this trial is to assess the effects of Aldesleukin administration on endogenous beta-cell function as measured by fasting blood sugar tests and postprandial C-peptide levels (Marcovecchio et al., 2020). A similar trial (NCT02411253) (Iltoo Pharma, 2015) to evaluate the efficacy of low-dose IL-2 for preserving residual pancreatic beta cell function was launched in 2015, estimated to complete in November 2022. Iltoo Pharma is conducting this double-blind randomized controlled parallel assignment study in France. So far, 141 participants, 6 to 35 years old, with detectable C-peptide level (> 0.2 pmol/ml) have been recruited. But no update has been posted (Iltoo Pharma, 2015).

Dendritic Cells Therapy Studies. Dendritic cells are another type of immune cell that can potentially prevent beta cell destruction in type 1 diabetes. A clinical trial (NCT02354911) was launched in 2015 by Diavacs, Inc. in collaboration with S. West Penn Allegheny Health to determine whether genetically modified autologous dendritic cells can serve as modulators of the immune system that disrupts the autoimmune process responsible for the destruction of pancreatic beta cells in subjects with new-onset type 1 diabetes (Diavacs, 2015). This study was a double-blind, placebo-controlled crossover study designed to evaluate the safety and efficacy of autologous immunoregulatory dendritic cells in 24 12- to 35-year-old patients with recent-onset type 1 diabetes (<100 days from diagnosis). The dendritic cells were collected via leukapheresis, incubated with antisense DNA oligonucleotides, and then injected back into the same subjects. Four injections were administered at 2-week intervals. At the end of 12 months, all subjects crossed over to the alternative treatment and continued to be followed for an

additional 12 months of therapy. So far, dendritic cell therapy has successfully controlled glucose in patients, and Diavacs received FDA approval to start phase II of the trial (Diavacs, 2015; The American Journal of Managed Care, 2014).

A second clinical trial with dendritic cell therapy started in 2019. This is a phase I/II double-blind (NCT03895996), randomized, placebo-controlled study of safety, tolerability, and potential efficacy of AVT001started by Avotres in 24 patients with recent onset (<100 days from diagnosis) type 1 diabetes, 16 years and older. AVT001 is an autologous dendritic cell-based drug that was developed to address the common root cause of autoimmune diseases by targeting the novel Qa-1/HLA-E restricted CD8+ regulatory T cell (Q/E CD8+ Treg) mediated pathway and regenerating its immunosuppressive properties. The study is still ongoing and is estimated to finish in 2023 (Avotres Inc., 2019).

Proinsulin and Proinsulin Hormone Gene Therapy Studies. Currently, genetically engineered proinsulin hormone and proinsulin gene are being investigated for safety and efficacy. The researchers hypothesize that the loss of tolerance to insulin likely contributes to the immunopathogenesis of type 1 diabetes; therefore, increasing tolerance to insulin has the potential to solve this problem (Precigen Actobio, 2021; Roep et al., 2013).

In 2018, Precigen ActoBio launched a clinical trial (NCT03751007) to study the safety and efficacy of AG019, a drug that delivers the autoantigen human proinsulin (hPINS) and the tolerance-enhancing cytokine human interleukin-10 (hIL-10) to the mucosal lining of the gastrointestinal tissues. This randomized, sequential study consists of two phases: phase Ib and phase IIa. PhaseIb is the open-label part of the study investigating the safety and tolerability of two different doses of AG019 where in two age groups (18-40 years of age and 12-17 years of age) in an open-label fashion. Phase IIa is the double-blind part of the study that is investigating

the safety and tolerability of AG019, in association with Teplizumab (a monoclonal antibody drug) in two age groups (18-40 years of age and 12-17 years of age) (Precigen Actobio, 2018). The study has yet to complete. However, interim results were shared via a press release stating that AG019 was well tolerated as a monotherapy and in combination with Teplizumab. Additionally, the researchers reported that both AG019 monotherapy and AG019 combination therapy resulted in stabilization of HbA1c and C-peptide levels and reduced conventional T-cells with an inflammatory phenotype. The researchers emphasized that the monotherapy results suggest that AG019 has the potential to be a standalone therapeutic agent for type 1 diabetes (Precigen Actobio, 2021).

Tolerion Inc. (formerly known as Bayhill Therapeutics) conducted a clinical trial (NCT00453375) from 2006 to 2011 to determine the safety and pharmacodynamics of TOL-3021. TOL-3021 is an engineered DNA plasmid encoding proinsulin. In this randomized, double-blinded, placebo-controlled study, 80 subjects over 18 years of age who were diagnosed with type 1 diabetes within the past 5 years were randomized in groups of 2:1 ratio to receive intramuscular injections of TOL-3021 (formerly labeled as BHT 3021) or placebo, once a week for 12 weeks. Four dose levels (0.3, 1.0, 3.0, and 6.0 mg) of TOL-3021 were evaluated in this study. No serious adverse events related to the drug were reported. C-peptide levels improved relative to placebo at all doses, demonstrating that a plasmid encoding proinsulin can preserve or even improve beta cell function in type 1 diabetes patients over the course of dosing (Roep et al., 2013).

Tolerion, Inc. launched another clinical trial (NCT03895437) in 2019 to further evaluate the efficacy of TOL-3021 in patients with new onset or established type 1 diabetes. With this study, the researchers hope to prove that TOL-3021 gene therapy product will completely halt

the autoimmune destruction of insulin-producing cells in patients and disease progression in type 1 diabetes patients. This study is a prospective multicenter, randomized, double-blind, placebo-controlled trial in 99 subjects aged 12 to 41 years within 5 years of type 1 diabetes diagnosis (first day of insulin administration). The subjects will be randomized in a 2:1 ratio to treatment with TOL-3021 or placebo for 52 weeks. Continuous glucose monitoring (CGM) devices will be initiated in subjects 5 days before the trial and continued through Week 52. In addition, C-peptide levels will be measured at weeks 12, 16, 24, and 52. The trial is currently ongoing and is estimated to complete in 2023 (Tolerion, 2019).

Monoclonal Antibody Treatments Studies. Monoclonal antibodies can stimulate and strengthen one's immune system, so the body is strong enough to fend off many diseases on its own. So far, monoclonal antibody treatments targeting interleukin cells have been found to be a successful form of antibody gene therapy in treating cancer, Crohn's disease, ulcerative colitis, plaque psoriasis, and psoriatic arthritis (Benson et al., 2011). The researchers hypothesized that monoclonal antibodies might be helpful in treating type 1 diabetes. Two studies investigated the safety and efficacy of monoclonal antibody drugs, Teplizumab and Ustekinumab, for type 1 diabetes (Herold et al., 2019; Marwaha et al., 2022). The clinical trial (NCT01030861) on Teplizumab was launched in 2010 by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) in collaboration with the American Diabetes Association. The randomized, placebo-controlled, double-blind trial was completed in 2019. The study included 76 participants, 8 to 45 years old, close relatives of type 1 diabetes patients, and at high risk for developing type 1 diabetes but had not been diagnosed with the disease at the start of the trial. The subjects were randomly assigned to a single 14-day Teplizumab or placebo course and then tested at 6-month intervals with oral glucose-tolerance tests to check for progression into type 1

diabetes. At the end of the trial, the researchers found that the median time of diagnosis with type 1 diabetes was 48.4 months in the Teplizumab group and 24.4 months in the placebo group. Additionally, the disease diagnosis was lower in the Teplizumab group than in the placebo group (43% vs. 72%). The researchers concluded that Teplizumab delayed progression to clinical type 1 diabetes in high-risk participants by about 2 years and cut the number of onsets by half (Herold et al., 2019). Teplizumab is currently under review by the United States Food and Drug Administration and is expected to receive approval by November 17, 2022. If approved, this will be the first disease-modifying therapy for type1 diabetes (ProventioBio, 2022).

Another monoclonal antibody drug, Ustekinumab or Ustekinumab, sold under the brand name Stelara, is currently being investigated for its efficacy in type 1 diabetes. Stelara targets interleukin-12 and interleukin-13 and therefore has the potential to halt type 1 diabetes disease progression. In 2015, the Juvenile Diabetes Research Foundation at the University of British Columbia launched a pilot clinical trial (NCT02117765) to investigate if Ustekinumab can protect and regenerate insulin-producing cells so that the affected individuals may be insulin free or require less insulin. In this study, 20 patients aged 18-35 years with recent onset of type 1 diabetes (<100 days of diagnosis) were sequentially enrolled into four subcutaneous dosing cohorts. Cohort A received Ustekinumab 45 mg on 0, 4, 16, 28, and 40 weeks, cohort B received Ustekinumab 90 mg on 0, 4, 16, 28, and 40 weeks, cohort C received Ustekinumab 45 mg on 0,4 and 16 weeks, and cohort D received Ustekinumab 90mg on 0, 4, and 16 weeks. The researchers found that the 90 mg groups showed the smallest mean decline in C-peptide level, demonstrating that the patients in this group experienced the least number of insulin-cell destruction. Therefore, this drug can potentially stop the progression of diabetes in recently diagnosed type 1 diabetes patients (Marwaha et al., 2022).

After the success of the pilot study, the researchers at the Juvenile Diabetes Research Foundation launched another trial (NCT03941132) in 2019 to investigate Ustekinumab further. In this study, the investigators plan to perform a phase II/III clinical trial with a total of 66 adult (18-35 years old) subjects with recent-onset type 1 diabetes (<100 days of diagnosis). There will be two study cohorts, with a drug to placebo ratio of 2:1. Patients in the treatment group will receive a loading dose of 6 mg/kg of Ustekinumab. After that, 90 mg of Ustekinumab, a total of seven doses will be given at weeks 8, 16, 24, 32, 40, and 48. The placebo group will receive respective amounts of a saline placebo. There will be 10 study visits over 78 weeks, three of which will be non-dosing and follow-up visits. During the non-dosing visits, patients' C-peptide levels will be measured. Patients will be followed for 78 weeks following the first dose. The study is expected to complete in 2024 (Juvenile Diabetes Research Foundation & Janssen, 2019).

Mesenchymal Stem Cells Studies. Mesenchymal stem cells are multipotent stem cells has the potential to be genetically modified or genetically enhanced to protect against type 1 diabetes progression (Kim et al., 2019). Currently, trials are ongoing to determine the efficacy of these cells on type 1 diabetes. One of these trials (NCT04061746) was launched by the Medical University of South Carolina in 2019 and is estimated to finish in 2026. This is a randomized, placebo-controlled, double-blind study with 60 participants, 18 to 30 years of age, with type 1 diabetes. These trials' primary and secondary outcome measure is C-peptide levels to indicate beta cell function (National Institute of Diabetes and Digestive and Kidney Diseases, 2019). The other trial (NCT05308836) was launched in 2021 by Vinmec Research Institute of Stem Cell and Gene Technology to evaluate the safety of intravenously administered adipose-derived mesenchymal stem cells in 10 patients 5 years and older with type 1 diabetes. The trial is estimated to complete in 2023. The primary outcome measure for the drug is safety, and the

secondary outcome measure is HbA1c, blood glucose, and C-peptide levels (Gwoxi Stem cell applied technology, 2021).

Antigen-Specific Treatment Studies. An antigen refers to a substance recognized by the body as a foreign substance and triggers an immune response to destroy that substance. With autoimmune disorders such as type 1 diabetes, the human body mistakenly recognizes its cells, especially pancreatic beta cells, as foreign substances and destroys them. Antigen-specific treatments induce the body to tolerate its cells, thereby preventing destruction. In May of 2022, the University of Colorado at Denver, in collaboration with Nova Immunotherapeutics Limited, launched an antigen-specific clinical trial with MER3101, an adjuvanted antigen-specific immunotherapeutic for the prevention and treatment of type 1 diabetes. MER3101 is a formulation of insulin B chain with MAS-1 adjuvant. An adjuvant is a substance that stimulates the immune system, so the immune system reacts more strongly to an antigen. The insulin B chain is part of an insulin molecule. This is a randomized, double-blind, placebo-controlled, phase 1 clinical trial with the primary objective to determine if MER3101 is safe and favors tolerogenic pathways to restore immunologic balance and reverse type 1 diabetes autoimmunity. For this study, 28 participants with recent-onset diabetes (>3 months), aged 18 to 45, were recruited. The safety and tolerability of three doses of progressively higher insulin B chain antigen doses at two doses (0.25 and 0.5 mL) will be tested. The study is estimated to complete in 2023 (University of Colorado, 2022).

T Regulatory Cells for Glucose Stabilization. Regulatory T cells or commonly known as Tregs, are a specialized subset of T cells that function to control the immune response and have the potential to stabilize type 1 diabetes. Preclinical studies in non-obese diabetic mice demonstrated that adoptive transfer of Tregs can slow diabetes progression and, in some cases,

reverse new-onset diabetes. Bluestone et al. at the University of San Francisco launched an open-label interventional clinical trial (NCT01210664) in 2010 to investigate the effect of T regulatory cells in humans. Fourteen participants aged 18 to 45 with type 1 diabetes were enrolled, where the patient's own T regulatory cells were isolated, genetically enhanced, and expanded to have superior functional activity. The patients were divided into four cohorts and received the T regulatory cells in a single infusion. The first cohort received 0.05 x 10^8 cells, the second cohort received 0.4 x 10^8 cells, the third cohort received 3.2 x 10^8 cells, and the fourth cohort received 26 x 10^8 cells. The subjects were followed for five years to assess the safety of the Treg therapy. The study was completed in 2015. No participants showed any adverse reaction to the Treg infusion, and the authors concluded that Treg cell infusions for type 1 diabetes are safe. The C-peptide levels were generally unchanged at 1 year and even after 2 years in dose cohorts 1 and 2. Additionally, the HbA1c level remained stable in all but one participant. The study results indicated that Treg cell infusion has the potential to stabilize type 1 diabetes (Bluestone et al., 2015).

However, most of the infused Tregs were undetectable in the peripheral blood three months after infusion. Therefore, the researchers started another clinical trial (NCT02772679) in 2016, combining polyclonal Tregs and low-dose interleukin (IL-2), to investigate if IL-2 affects Treg survival and expansion in the bloodstream. Sixteen patients aged 18 to 45 with type 1 diabetes were treated with a single infusion of autologous polyclonal Tregs followed by one or two 5-day courses of recombinant human low-dose IL-2 (ld-IL-2). The combination therapy led to an increase in the number of infused and endogenous Tregs along with a substantial increase in the number of cytotoxic cells. Unfortunately, the combination therapy did not stabilize the C-peptide level, which decreased dramatically at 28 days of treatment. The Data and Safety

Monitoring Board (DSMB) expressed their concern over this decrease, recommended altering the IL-2 dose, and ultimately advised termination of the study because of the low likelihood that clinical benefits such as beta cell preservation could be achieved with the Treg and IL-2 combination (Dong et al., 2021).

Another study (NCT02691247) was started by Caladrias Bioscience, Inc. in collaboration with the California Institute of Regenerative Medicine in 2016 to evaluate the safety and efficacy of Treg cells in adolescents. This trial was a prospective randomized placebo-controlled double-blind clinical using CLBS03, an infusion of autologous ex vivo expanded polyclonal regulatory t-cells in 113 participants, 8 to 17 years old, with recent-onset type 1 diabetes (<100 days of diagnosis). The study was estimated to complete in 2020; however, no results have been posted thus far (California Institute for Regenerative Medicine, 2016). In 2019, Caladrius Bioscience shared interim results through a press release stating that CLBSO3 was well tolerated at the one-year follow-up at doses of 2.5 million cells/kg or 20 million cells/kg but the efficacy of CLBSO3 on the preservation of C-peptide levels in the treatment group was not significantly higher than the placebo group (Caladrius Biosciences, 2019).

T Regulatory Immunotherapy in Islet Transplantation Studies. The University of Alberta, in collaboration with various diabetes research foundations, is investigating the effect of T regulatory cells on the immune systems of the islet transplant recipients. Patients who receive transplants must take lifelong immunosuppressive medication to suppress their immune system and prevent rejection of their transplant tissues/organs. However, even with a rigorous immunosuppressant regimen, rejection of the islets continues, and most patients need at least two transplant sessions to achieve complete insulin independence. Additionally, the immunosuppressants make the patients susceptible to infections and increase morbidity in those

patients. Preclinical studies demonstrated that Regulatory T cells (Tregs) could contribute to self-tolerance by preventing the initiation of unwanted immune activation and by suppressing ongoing immune responses to limit bystander tissue destruction. Therefore, the researchers hypothesized that the infusion of Tregs before extensive graft damage might improve long-term graft outcomes and launched two clinical trials.

The first clinical trial (NCT03444064) started in 2018 and is estimated to finish in late 2022. This trial will assess the safety and feasibility of intravenous infusion of ex vivo-selected and ex vivo-expanded autologous PolyTregs in islet transplant patients and the effect of Tregs on beta cell function in islet transplant patients. Eighteen participants, 18 to 68 years old, were recruited for this study; six were assigned to a control group and 12 to an intervention group. After transplantation, both groups will receive the current Edmonton islet transplant induction therapy. The intervention group will receive PolyTregs (400-1600 million) six weeks post-transplant and will be followed for one year to assess the safety and preliminary efficacy of Treg therapy (Diabetes Research Institute, 2018). The second trial (NCT05349591) will start in late 2022 to investigate the effect of ex-vivo expanded autologous cryopreserved polyclonal regulatory t cell (cePolyTregs). Cryogenically preserved cells are more convenient than freshly collected cells. Therefore, the researchers are interested in discovering if cryogenically preserved PolyTregs are just as good as freshly collected PolyTregs. This study will have an exact study design as the first one but one difference. In this study, the treatment group will receive cePolyTregs instead of freshly collected PolyTreg cells (University of Alberta, 2022a).

Xenotransplantation Studies. Four clinical trials are investigating xenotransplantation for type 1 diabetes. These studies were conducted with the primary objective to investigate if porcine islets could be used safely and effectively instead of human pancreatic islets as donor

human pancreatic islets are scarce. Three of these trials (NCT00940173, NCT01739828, NCT01739829) were conducted by Diatranz Otsuka Ltd. (formerly known as Living Cell Technologies), and one was conducted by Hunan Xeno-life Science Ltd. (NCT03162237) (Cooper et al., 2016; Hunan Xeno-life Science, 2013).

Diatranz Otsuka Ltd. used porcine islets taken from designated pathogen-free animals bred in isolation and monitored to be free of specified pathogens to avoid any xenotic infection (the transmission of infectious agents between species via xenograft). To protect against immune rejection, researchers at Diatranz Otsuka encapsulated porcine cells in alginate microcapsules. These specialized porcine cells are called DIABECELL, designed to avoid detection as a foreign agent by the body while permitting the inward passage of nutrients and glucose and the outward passage of insulin. After preclinical animal studies confirmed the safety and efficacy of DIABECELLs, Diatranz Otsuka Ltd. conducted its first human trial (NCT00940173) from 2009 to 2014 in Auckland, New Zealand. Encapsulated neonatal porcine islets were laparoscopically implanted into the peritoneal cavity of 14 type 1 diabetes patients, ages 35 to 65, without any immunosuppressive drugs. The patients received encapsulated islet equivalents of 5,000 (n = 4; group 1), 10,000 (n = 4; group 2), 15,000 (n = 4; group 3), or 20,000 (n = 2; group 4) per kg body weight. After transplantation, the number of unaware hypoglycemia events was reduced in all groups, and four out of 14 patients attained HbA1c <7%. There were no xenotic infections or serious adverse events; only hypersensitivity, post-procedural discomfort, anxiety, and depressed moods were reported in some patients. Therefore, the researchers concluded that encapsulated porcine islets were safe for transplantation (Cooper et al., 2016; Matsumoto, Tan, Baker, Durbin, Tomiya, Azuma, Doi, et al., 2014).

Diatranz Otsuka Ltd. conducted two other trials (NCT01736228, NCT01736229) in Buenos Aires, Argentina, from 2011 to 2014. Two doses, approximately 5000 IEQ/kg and 10,000 IEQ/kg of encapsulated neonatal porcine islets were transplanted twice, 12 weeks apart, in four type 1 diabetic patients in each group. In the higher dose group, all four patients showed improved HbA1c, which was maintained at a level of < 7% for more than 600 days with a significant reduction in the frequency of unaware hypoglycemic events. In addition, there were no xenotic infections or severe adverse events experienced by the patients (Cooper et al., 2016; Matsumoto et al., 2016).

Hunan Xeno-life Science Ltd. conducted the last study. This was an open-label randomized parallel assignment in 2013 where 20 patients, ages 18 to 40, received neonatal porcine islets. The patients also received autologous T regulatory cells to induce tolerance and avoid rejecting the newly grafted porcine cells. According to the clinical trials site, the trial finished in 2018; however, no results have been published thus far (Hunan Xeno-life Science, 2013).

Type 2 Diabetes

Six studies were found that performed gene therapy for type 2 diabetes. Two of these studies used a cell-based gene therapy approach using mesenchymal stem cells (Ukraine Association of Biobank, 2020; Vinmec Research Institute of Stem Cell and Gene Technology, 2017), three used glucokinase or GCK gene therapy for type 2 diabetes (AstraZeneca, 2020), and one used p53 gene therapy (Shenzhen SiBiono GeneTech Co., 2015).

The cell-based gene therapy approach using mesenchymal stem cells has shown to be a promising strategy for providing safe, targeted, and efficient gene delivery. In 2020, the Ukraine Association of Biobank started a multicenter, long-term safety and efficacy follow-up study

(NCT04642911) for insulin-dependent type 2 diabetes patients who will be treated with ex vivo gene therapy using mesenchymal stem cell products AUB001. This trial is a 10-year study where a patient pool consisting of 91 children, adults, and older adults will complete a 2-year treatment and will be followed for years to measure the overall survival of subjects with diabetes with AUB001. The estimated completion date of this study is October 10, 2030 (Ukraine Association of Biobank, 2020). So far, no updates have been posted on the clinical trials site. The trial has likely been impacted by the current war in Ukraine (QPS, 2022).

Bone marrow-derived mesenchymal stem cells have the potential to provide immunosuppressive effects by secreting a variety of cytokines (small protein molecules crucial in cell signaling and immune action) targeting insulin resistance tissues (Kim et al., 2019). Vinmec Research Institute of Stem Cell and Gene Technology in Hanoi, Vietnam, conducted a randomized controlled, open-label study (NCT03343782) from 2017 to 2019 to evaluate the safety and effectiveness of autologous bone marrow-derived mesenchymal stem cell transplantation for type 2 diabetes. For this study, 30 patients 18 years or older with type 2 diabetes for more than 5 years were enrolled. The safety of the treatment was evaluated by the number of adverse events, and the efficacy was evaluated by the absolute changes in the HbA1c, fasting blood glucose, and C-peptide levels throughout the 12-month follow-up. There were no serious adverse events related to the treatment. Data collected during the study and the follow-up period indicated that autologous bone marrow-derived mesenchymal stem cell administration was well tolerated in all 30 patients. In addition, the researchers found out that patients with diabetes duration of less than 10 years and a body mass index of under 23 kg/m^2 showed a significant reduction in their HbA1c levels and moderate reduction in fasting blood glucose levels and C-peptide levels during their 3-, 6-, and 9-month follow-up and experienced normal

fasting blood sugar levels (6.8 ± 2.4 mmol/L) at the end of the 12-month study period. Other patients with diabetes duration of more than 10 years and body mass index over 23 kg/m^2 demonstrated reductions in HbA1c levels and fasting blood sugar levels for the first three months; however, the pre-treatment levels of HbA1c and fasting blood sugar levels gradually returned after six months. The researchers concluded that autologous bone marrow-derived mesenchymal stem cell transplantation is only effective in patients with type 2 diabetes duration of fewer than 10 years and with a body mass index under 23 kg/m^2 (Nguyen et al., 2021).

In 2015, Shenzhen SiBiono GeneTech started a phase II clinical trial (NCT02561546) with p53gene therapy (drug name Gendicine) for treating diabetes concurrent with hepatocellular carcinoma (Shenzhen SiBiono GeneTech Co., 2015). This study's objective was to investigate the anti-diabetic and anti-tumor roles of p53 gene therapy. This trial was an open-labeled, randomized, active-controlled phase II study where the p53 gene was administered via the artery that supplied blood for the tumor nodules. The endpoints for the anti-diabetic role were fasting plasma glucose, postprandial glucose, and HbA1c at 30 days after the start of treatment; anti-tumor effects are progression-free survival and overall survival. The study was estimated to complete in 2017. However, no update was posted on clinicaltrials.gov site. Nevertheless, the researchers briefly mentioned in an article published in *Human Gene Therapy* journal that treating hepatocellular carcinoma using p53 gene therapy controlled concurrent diabetes and lowered the patient's blood sugar levels, allowing them to use insulin less frequently. The researchers also reported that this positive effect persisted for more than a year after Gendicine treatment (Zhang et al., 2018).

AstraZeneca conducted several studies with glucokinase activators in 2008. Glucokinase in the pancreas serves as a glucose-sensor and elicits insulin secretion (Kiyosue et al., 2013).

Therefore, activating glucokinase in the pancreas could stimulate insulin secretion and lower blood sugar levels in people with diabetes. In 2010, AstraZeneca conducted a randomized, double-blind, placebo-controlled, phase II clinical study (NCT01152385) to investigate the safety, tolerability, and efficacy of AZD-1656, a glucokinase activator. The study took place in Japan; 224 patients with type 2 diabetes, 30 to 65 years old, received treatment with high (200 mg/day), medium (140 mg/day), or low-dose (80 mg/day) AZD-1656 or placebo. After 2 months, the levels of HbA1c in all treatment groups decreased by 0.3–0.8% compared to the placebo group (0.1%.). Unfortunately, at 4 months of treatment, the reduction in HbA1c in the treatment group was found to be similar to the placebo group, indicating that the effectiveness of AZD -1656 tapered off over time. This trial demonstrated that AZD-1656 significantly lowered blood sugar levels in the short-term but not in the long-term. In 2011, AstraZeneca terminated the AZD-1656 program in Japan due to unsatisfactory results (Kiyosue et al., 2013).

AstraZeneca conducted a second glucokinase activator study with AZD-6370 in Sweden around the same time. This was a dose-finding, randomized, single-blind, placebo-controlled, crossover study (NCT00690287) in eight patients aged 35 to 75 with type 2 diabetes. The study was divided into two parts. For part A, 16 patients received a single oral dose of AZD-6370 (20, 60, or 180 mg) or placebo in the fasted or fed states. In Part B, eight patients received a placebo and a total dose of AZD-6370 180 mg given in one, two, or four divided doses. Plasma glucose, insulin, and C-peptide changes versus placebo were assessed within 24 hours of treatment. AZD-6370 provided dose-dependent reductions in plasma glucose of up to 30% versus placebo in both fasted and fed patients. Insulin secretion was found to have a dose-dependent relationship with AZD-6370 and increased with dose (Ericsson et al., 2012).

AstraZeneca is currently conducting a third glucokinase activator study in England. This study (NCT05216172) is a single-site, placebo-controlled, double-blind, randomized clinical trial of AZD1656 in patients with type 2 diabetes who have received a new renal transplant. Transplant recipients with pre-existing type 2 diabetes frequently experience high blood sugar levels due to the immunosuppressants they are required to take after transplantation. Unfortunately, these high blood sugar levels cause poor transplantation outcomes. For this study, 50 patients 18 years and older were randomized to receive a 3-month course of either active drug or placebo within 24 hours of transplantation. The researchers plan to collect clinical and laboratory data and assess at baseline and throughout their participation in the study. The trial began in early 2020 and is estimated to complete in late 2022 (AstraZeneca, 2020).

Diabetes-Related Complications

Neuropathy Studies. Five studies focused on diabetic neuropathy, a condition that causes a loss of sensation due to diabetic nerve damage (Ajroud-Driss et al., 2013; Ajroud-Driss et al., 2015; Kessler et al., 2021; Kessler et al., 2015; Ropper et al., 2009). From 2002 to 2008, Losgordo et al. conducted a randomized, double-blind, placebo-controlled clinical trial (NCT00056290) to evaluate vascular endothelial growth factor (VEGF) for diabetic polyneuropathy. The researchers discovered in preclinical studies that VEGF, a genetic material injected into the leg muscles of the affected leg, could help new blood vessels to grow. In this study, plasmid VEGF was used to treat polyneuropathy. Fifty patients, ages 21 and older, with polyneuropathy were randomized. Thirty-nine patients received VEGF, and 11 received a placebo. Three sets of injections were given at eight standardized sites adjacent to the sciatic, peroneal, and tibial nerves of one leg. Mean symptom score improved in both legs at 6 months, favoring VEGF over placebo (–1.2 ± 0.5 vs. –0.9 ± 0.5) after adjustment for change in the

untreated leg) and compared to the untreated leg (−0.7 ± 0.5). The region of sensory loss and pain level improved in the treated group (−1.5 vs. −0.5). However, there were 84 adverse events reported in the treatment group, and 22 were considered severe. Therefore, the researchers concluded that while the intramuscular plasmid VEGF gene transfer improved diabetic neuropathic symptoms in the treated group, the number of adverse events was too high (Ropper et al., 2009).

The other trials were conducted by Helixmith Co., Ltd. (Ajroud-Driss et al., 2013). These trials were performed to evaluate VM202, a plasmid DNA encoding two isoforms of hepatocyte growth factor (HGF) for diabetic polyneuropathy. A phase I/II open-label dose-escalation study (NCT01002235) was conducted to assess the safety and tolerability of VM202 in patients with painful diabetic peripheral neuropathy. The study took place from 2010 to 2012. Twelve patients, 18 to 75 years old, were placed in three dose cohorts (4, 8, and 16 mg). All cohorts received two sets of VM202 injections separated by 2 weeks. Levels of pain were measured throughout 12 months after treatment with a visual analog scale (VAS: general pain rating scale), the short form McGill questionnaire (SF-MPQ: type of pain rating scale), and the brief pain inventory for patients with diabetic peripheral neuropathy (BPI-DPN: pain rating scale specific to diabetic peripheral neuropathy). There were no adverse events related to treatment. The mean VAS was reduced from baseline by 47.2% at 6 months and 44.1% at 12 months after treatment. Additionally, the VAS scores for the 4, 8, and 16 mg dose cohorts at 6 months follow-up decreased in a dose-responsive manner by 21%, 53%, and 62%, respectively. Pain measured by other scales, SF-MPQ and BPI-DPN, demonstrated a similar reduction of pain in patients treated with VM202. Therefore, it was concluded that VM202 could be a safe, well-tolerated, and

effective treatment for long-term symptomatic relief and better quality of life in patients with peripheral diabetic neuropathy (Ajroud-Driss et al., 2013).

Helixmith Co. conducted the phase II part of the safety and efficacy of the VM202 clinical trial (NCT01475786) from 2012 to 2014. One hundred and four patients, 18 to 75 years old, with peripheral neuropathy, were enrolled in this double-blind, randomized, placebo-controlled, multicenter study. All patients were randomized to receive injections of 8 or 16 mg VM202 per leg or placebo. Those who took gabapentin or pregabalin for nerve pain were allowed to continue taking them during the trial. Divided doses were administered on Day 0 and Day 14. There were no significant adverse events related to the treatment. Eighty-four patients completed the study. Of these patients, 48.4% experienced a ≥50% reduction in pain compared to 17.6% of placebo patients. Patients receiving 8 mg VM202 per leg showed the most improvement in all efficacy measures, including a significant reduction in the mean pain score at 3 months. The mean pain score continued to decrease over time but was not as significant at 6 and 9 months. Pain scores measured by the Pain Inventory for Patients with Diabetic Peripheral Neuropathy and the Michigan Neuropathy Screening Instrument reflected similar pain reductions in treated patients. Interestingly, patients who were not taking pregabalin or gabapentin reported the most significant reductions in pain. The study showed that VM202 was safe, well tolerated, and so effective that only two days of treatment were sufficient to provide symptomatic relief and improve quality of life for 3 months. In addition, VM202 may be most beneficial for patients who are not on gabapentin or pregabalin for diabetic polyneuropathy (Ajroud-Driss et al., 2015).

Helixmith Co. conducted the phase III part of the safety and efficacy of the VM202 from 2016 to 2019. This double-blind, placebo-controlled, multicenter study had two parts: DPN 3-1 (NCT02427464) and DPN 3-1b (NCT04055090). For DPN 3-1, 500 subjects were randomized

and placed in a 2:1 ratio where 336 participants received VM 202 and 164 received placebo for 9 months, with the primary objective to measure change from baseline in the mean 24-hour Numerical Rating Scale (NRS) pain score. VM202 or placebo was administered to calf muscles on days 0 and 14 and on days 90 and 104. DPN 3-1 ended on day 270, and a preplanned subset of 101 subjects (VM202: 65 subjects; and placebo: 36) entered the noninterventional extension, DPN 3-1b, until day 365. VM202 was well-tolerated in both parts of these trials without significant adverse events. The VM202 treatment group did not meet its pain reduction efficacy end points in DPN 3-1 but reduced pain significantly during DPN 3-1b. Pain reduction in DPN 3-1b was even more remarkable in subjects not receiving gabapentin or pregabalin, confirming an observation noted in the phase II part of the study. In DPN 3-1b, symptomatic relief was maintained for 8 months after the last injection suggesting that VM202 treatment in DPN 3-1 might have changed disease progression and reduced pain even when the patients did not receive treatments. The researchers plan to conduct another phase III trial to confirm if VM 202 can stabilize or reverse diabetic polyneuropathy with just one or two treatments (Kessler et al., 2021).

Critical Limb Ischemia Studies. Seven studies were found on gene therapy for critical limb ischemia complicated by diabetes mellitus (Barć et al., 2021; Belch et al., 2011; Cui et al., 2015; Grossman et al., 2016; Gu et al., 2019; Gu et al., 2011; Hammad et al., 2020; Kusumanto et al., 2006). Critical limb ischemia, a prevalent and dangerous disease, represents the ultimate stage of peripheral arterial disease, defined by constant rest pain, ulceration, and gangrene leading to amputation (Simon et al., 2022). Gene therapy trials for critical limb ischemia are being conducted to reduce constant pain, ulceration, gangrene, and amputations. Kusumanto et al. (2006) led a clinical trial to assess the efficacy of intramuscular administration of

phVEGF165 and reduce amputation rate at 100 days of administration. phVEGF165 is a vascular endothelial growth factor gene-carrying plasmid. The trial was a two-center, randomized, double-blind, placebo-controlled study in the Netherlands that lasted from 2000 to 2004. Fifty-four diabetic patients, 18 to 85 years old with critical limb ischemia were randomized, then 27 were placed in the treatment group to receive 2000 micrograms of phVEGF165, and the other 27 patients were placed in the placebo group to receive 0.9% NaCl on day 0 and day 28. Clinical symptoms wound status, and hemodynamic (blood circulation) status were assessed on days 7, 14, 35, 42, 72, and 100. The treatment failed to reach its primary goal of completely eliminating the number of amputations; however, it reduced the incidences by half (3 vs. 6 in placebo). Additionally, it significantly improved hemodynamics (33% vs. 6% in placebo), skin ulcers (33% vs. 0% in placebo), and pain (24% vs. 18% in placebo) without any significant adverse events (Kusumanto et al., 2006).

Gu et al. (2011) conducted a clinical trial in China to evaluate the safety, tolerability, efficacy, and appropriate dose of a plasmid DNA therapy expressing two isoforms of hepatocyte growth factor NL003 (pCK-HGF-X7) in critical limb ischemia patients. The trial had two phases, I and II; phase I took place from 2008 to 2009. Twenty-one subjects with critical limb ischemia were consecutively assigned to receive increasing doses where cohort I received 4 mg, cohort II received 8 mg, cohort III received 12 mg, and cohort IV received 16 mg of NL 003 in the ischemic calf and thigh muscle at days 1 and 15. A safety and tolerability evaluation and measurement of pain severity score using a visual analog scale (VAS), ulcer status, transcutaneous oxygen (TcPO2:measure of blood circulation), and ankle-brachial index (ABI: ankle blood pressure) were performed throughout a 3-month follow-up period. No serious adverse events were reported. A significant reduction in pain was observed in treated patients;

the mean VAS value of all patients decreased from 4.52 at baseline to 0.30. The mean ABI value increased from 0.49 at baseline to 0.63 at the 3-month follow-up. The mean TcPO2 value also significantly improved at the 3-month follow-up. In addition, significant wound healing was observed in six of nine patients with an ulcer at baseline (Gu et al., 2011).

The phase II trial to assess the clinical safety and efficacy of intramuscular injection of NL003 in critical limb ischemia occurred from March 2012 to June 2014. The treatment portion of the trial lasted 6 months. The study recruited two hundred patients, classified as level 4 and 5 critical limb ischemia patients, by the Rutherford scale. These patients were randomly assigned to four cohorts to receive low-dose NL003, middle-dose NL003, high-dose NL003, or placebo. The treatments and placebos were administered on the affected limbs on days 0, 14, and 28. No significant adverse events were reported. No statistically significant differences in blood circulation or blood pressure were detected in the four cohorts. However, pain severity was significantly reduced in all NL003 groups compared to the placebo group at 6 months. Additionally, the proportion of patients with complete ulcer healing in the high-dose group was significantly higher compared to the placebo group. These results demonstrated that plasmid DNA therapy with hepatocyte growth factor NL003 could induce total healing of ulcers in treated legs, complete pain relief without painkillers safely in level 4 and 5 critical limb ischemia patients (Gu et al., 2019).

Gu et al. conducted another study around 2014 with a design similar to phase I of the NL003 trial but with a different plasmid (pUDK-HGF DNA) expressing human hepatocyte growth factor (HGF) in patients with critical limb ischemia. In this prospective, open-label, dose-escalation, single-center study, 21 patients were enrolled and randomly divided into four dose cohorts to receive 4, 8, 12, or 16 mg of pUDK-HGF, respectively. Each patient received local

intramuscular injections in the calf and thigh muscles twice, the first injection of half of the dose of pUDK-HGF on day 1, and the second half on day 15. Safety, including adverse events and physiological parameters, and preliminary efficacy, including pain severity score measured by visual analog scale (VAS), ulcer size and ankle-brachial index (ABI), and transcutaneous oxygen (TCPO2), were evaluated at baseline and throughout a 3-month follow-up period. The patients well-tolerated all doses of pUDK-HGF, and none of the adverse effects were related to the treatment. Two significant clinical results were observed after pUDK-HGF administration. The mean VAS value of all patients decreased from 4.52 at baseline to 0.30. Fourteen patients experienced a complete reduction of pain by day 91. Two of the four ulcers had completely healed, 25% ulcer size reduction. Of five patients with gangrene, one gangrenous wound had healed completely, and two showed marked size reduction by day 91. The mean blood circulation and blood pressure scores also improved (Cui et al., 2015).

A different type of growth factor, fibroblast growth factor or NV1FGF, a novel pCOR (conditional origin of replication) DNA plasmid-based gene delivery system, is in development by Sanofi-Aventis to treat critical limb ischemia (Maulik, 2009). Following intramuscular injection into an area of restricted blood flow, NV1FGF is taken up by muscle cells resulting in an increased expression of FGF protein, promoting blood vessel growth. From 2007 to 2012, Sanofi carried out an international, double-blind, placebo-controlled, randomized with 525 critical limb ischemia patients, 60-80 years old, 70% males, and 53% diabetic patients, from 170 sites worldwide. The patients were randomized and stratified by country and by diabetes status. Two hundred and fifty-nine patients were assigned to the intervention group to receive NV1FGF at 0·2 mg/mL and 266 to the placebo group to receive a visually identical placebo. All patients received eight intramuscular injections of their assigned treatment in the index leg on days 1, 15,

29, and 43. The primary endpoint was major amputation or death at 1 year. Unfortunately, the difference in the number of major amputation or death between the intervention group and the placebo group (86 vs. 96; 33% vs. 36%) were not significant at 1 year. Therefore, it was concluded that NV1FGF is not an effective treatment in reducing amputation or death in patients with critical limb ischemia (Belch et al., 2011).

Barć et al. (2021) at the Wroclaw Medical University in Poland conducted a study from 2017 to 2018 to evaluate the safety, feasibility, and clinical efficacy of gene therapy in patients with critical limb ischemia complicated by diabetes. Twenty-eight patients, ages 40 to 85, with a duration of diabetes ranging from 6.5 to 28 years, were recruited for the study. The patients were then randomized and placed into two groups to receive treatment (n = 14) or placebo (n = 14). The treatment group received an intramuscular injection of 4 mg plasmid pIRES/VEGF165/HGF bicistronic plasmid into the ischemic lower limb above and below the knee level at weeks 1, 4, and 12 weeks. Ninety days after administration, rest pain decreased significantly compared to the control group, and considerable improvement in vascularization was observed. Therefore, gene therapy with pIRES/VEGF165/HGF could be an effective treatment method for patients with critical limb ischemia complicated by diabetes (Barć et al., 2021)

MultiGene Vascular Systems Ltd. conducted a phase I, safety, dose-escalating, non-randomized, open-label study (NCT00390767) with MultiGeneAngio, a product developed for the treatment of patients with narrow or blocked arteries in the legs. MultiGeneAngio comprises endothelial and smooth muscle cells isolated from a short vein segment stripped from the patient's arm. After isolation, the cells are expanded, characterized, and genetically modified with the transfer of angiogenic genes. Then MultiGeneAngio is injected intra-arterially at the site of blockage using a standard diagnostic catheter to potentially create and expand new collateral

arteries, thereby improving blood flow to an ischemic limb. The trial took place from 2006 to 2010 in two centers, where 12 subjects, 50-80 years old, divided equally into four cohorts, received escalating doses of MultiGeneAngio 1 dose per patient in the first cohort, two doses per patient in the second cohort, five doses per patient in the third cohort and seven doses per patient in the fourth cohort, administered as an intra-arterial infusion. All subjects were male (mean age 60 ± 5 years) including 25% with diabetes mellitus. The primary outcome measures were clinical safety and tolerability. Other safety measures included ankle-brachial index (ABI) and walking time on a treadmill. At 1-year follow-up, there was one serious adverse event possibly related to the drug. Safety endpoints were within normal ranges in all subjects. The overall blood pressure (ABI) in the affected limb remained stable. The mean walking time increased from baseline to 1 year. It was concluded that MultigeneAngio was safe and well-tolerated in patients with critical limb ischemia (Grossman et al., 2016).

Juventas Therapeutics, Inc., from 2015 to 2017, carried out a double-blind, placebo-controlled trial (NCT02544204) to examine the impact of complementing revascularization therapy with intramuscular JVS-100 (stromal cell-derived factor-1 plasmid treatment), a nonviral gene therapy to activate endogenous regenerative repair pathways. One hundred and nine patients with critical limb ischemia were randomized to receive a placebo, 8 mg, and 16 mg JVS-100, respectively. The average patient age was 71 years; 33% of the patients were women, 79% had diabetes, and 8% had end-stage renal disease. The primary efficacy endpoint of the trial was a 3-month and 6-month wound healing score assessed by an independent wound core laboratory. The primary safety endpoint was major adverse limb events (amputation) at 3 and 6 months of treatment. Unfortunately, at 3 months of treatment, only 26% of wounds completely healed without significant differences between the three groups (26.5%, 26.5%, and 25%, respectively).

Three (2.8%) patients died, and two (1.8%) had major amputations. The significant adverse limb event rates at 3 months were 8.8%, 20%, and 8.3%, respectively. While safe, JVS-100 failed to improve wound healing or hemodynamic measures at 3 months (Shishehbor et al., 2019), one-third of the patients had complete wound healing at 6 months; 31% in the placebo group, 33% in the 8-mg injection group, and 33% in the 16-mg injection groups. Again, showing no significant difference between the placebo and the treatment groups. It was concluded that a combination of revascularization and gene therapy failed to improve outcomes for patients with critical limb ischemia in 6 months of JVS-100 treatment (Hammad et al., 2020).

Diabetes Related Eye Complications Studies. Four studies were found in diabetes-related eye complications. People with diabetes suffer from eye complications such as retinopathy, macular edema, and age-related macular degeneration. Diabetic retinopathy is a chronic and progressive complication of diabetes that threatens one's eyesight. Retinopathy is characterized by neuronal and vascular dysfunction in the retina in the early stages and by neovascularization that leads to loss of functional vision in the later stages (National Eye Institute, 2022b). REGENXBIO, Inc. started a randomized, dose-escalation, observation-controlled study (NCT04567550) in 2020 to evaluate the efficacy, safety, and tolerability of RGX-314 for diabetic retinopathy. RGX-314 is a gene therapy product that includes the NAV-AAV8 vector containing a gene encoding for a monoclonal antibody fragment designed to inhibit vascular endothelial growth factor (VEGF) (REGENXBIO, 2020). This study has a 3-arm design. The researchers plan to enroll approximately 60 participants into three cohorts where cohort I is to evaluate RGX-314 dose 1, and cohorts II and III are to evaluate RGX-314 dose 2. Cohort I completed the study on January 18, 2022, while enrollments were still ongoing for cohorts II and III. REGENXBIO, Inc. shared the cohort I results through a press release stating

that 47% of the patients in cohort I treated with RGX-314 demonstrated a two-step or greater improvement from baseline on the Early Treatment Diabetic Retinopathy Study-Diabetic Retinopathy Severity Scale (DRSS), compared to zero patients in the control group. They added that no drug-related serious adverse events occurred at six months of treatment and RX-314 seemed well-tolerated by patients in cohort I (REGENXBIO, 2022). The investigation with cohort II and III are still ongoing (REGENXBIO, 2020).

In 2020, Adverum Biotechnologies, Inc. started a phase 2, multicenter, randomized, open-label, active-controlled study (NCT04418427) of ADVM-022 in subjects with diabetic macular edema. ADVM-022 is an adeno-associated virus vector encoding aflibercept, a gene therapy product developed for treating serious retinal vascular diseases, including diabetic macular edema, which affects up to 10% of people with diabetes caused by fluid accumulation in the macula and is the most frequent cause of blindness in people with diabetes. Current therapies for retinal vascular diseases include laser treatment and frequent and long-term intravitreal injections of anti-vascular endothelial growth factor drugs. Unfortunately, the need for recurring treatments often leads to undertreatment, disease progression, and subsequent vision loss in patients with this disease. Most patients with macular edema would prefer a one-time anti-vascular endothelial growth factor administration. Therefore, Adverum Biotechnologies designed ADVM-022 to demonstrate superior control of diabetic macular edema with a single injection compared to a single aflibercept injection. For this trial, 36 participants were randomized to one of three cohorts where cohort I received a high dose (6 x 10^11 vg/eye) of ADVM-022, cohort II received a low dose (2 x 10^11 vg/eye) of ADVM-022, and cohort III received aflibercept (2 mg/eye). Patients assigned to receive ADVM-022 were further randomized to receive either a preceding aflibercept or sham ocular injection (Adverum Biotechnologies, 2020). All subjects

were to be followed for 96 weeks after randomization. Unfortunately, after a month of the treatment, three patients developed hypotony (low intraocular pressure) requiring surgical treatment, followed by a severe, progressive decline in vision. Additionally, all patients in the higher dose ADVM-022 experienced intraocular inflammation. Even though the patients in the low dose ADVM-022 experienced improved vision, the adverse events in the higher dose cohort were so severe that the trial was canceled (HCP Live, 2021).

Adverum Biotechnologies, Inc. conducted a different gene therapy trial for patients with age-related macular degeneration. This trial (NCT01494805) aimed to investigate the safety and efficacy study of rAAV.sFLT-1, a viral vector used to deliver a gene that expresses a therapeutic protein in the eye in patients with exudative age-related macular degeneration. This disease causes central vision loss and represents a significant health problem in older adults, especially those with diabetes (National Eye Institute, 2022a). The study was designed to take place in three3 stages: phase 1, phase 2a, and a 3-year follow-up (Rakoczy et al., 2019).

Phase I was a single-center, open-label, randomized controlled trial that took place in Australia from December 16, 2011, to April 5, 2012. Eight patients, 65 years and older, were randomly assigned to receive either intervention: three patients in the low-dose rAAV.sFLT-1 group and three patients in the high-dose rAAV.sFLT-1 group, and two patients in the control group. Patients in the intervention group received treatment at baseline and week 4. No drug-related adverse events were noted, and at the one1-year follow-up, it was concluded that rAAV.sFLT-1 was safe and well tolerated (Rakoczy et al., 2015). The phase II trial took place between August 2012 and March 2014 and was a single-center, open-labeled randomized controlled trial. Thirty-two patients, 55 years and older, were randomized; 21 were placed in the treatment group, and 10 were placed in the controlled group. The treatment group received

rAAV.sFLT-1 at day 7. All patients were assessed every 4 weeks to the week 52 primary endpoint, with a long-term follow-up to 36 months. No serious adverse events were reported, and any ocular adverse events (AEs) in the rAAV.sFLT-1 group were mainly procedure-related and self-resolved. Additionally, 12 of 21 (57%) gene therapy patients experienced maintenance or improvement of vision versus four of 11 (36%) control patients (Constable et al., 2016). The three-year follow-up demonstrated that gene therapy treatment with rAAV.sFLT-1 was well-tolerated among the patients. Those who responded well to the treatment demonstrated continual improvement during the 12- and 24-month follow-up visits. However, the researchers concluded that the study populations were too small to make any conclusive remarks on the efficacy of this drug (Rakoczy et al., 2019).

People with diabetic retinopathy or age-related macular degeneration are usually excluded from angiogenic gene therapy trials due to the fear that angiogenic gene therapy may negatively impact patients' retinal pathologies (Qazi et al., 2009). However, if these patients are always excluded from angiogenic gene therapy trials, finding appropriate gene therapy solutions for their ailments would be impossible. Prokosch et al. wanted to investigate whether angiogenic gene therapy causes retinal pathology in patients with diabetic retinopathy or age-related macular degeneration. The researchers conducted a retrospective subgroup analysis of 26 patients with diabetic retinopathy or age-related macular degeneration out of the 152 patients that participated in two phase II, multinational, double-blind, randomized, placebo-controlled angiogenic gene therapy trials (TALISMAN 201 and 211). The patients received up to 32 mg of nonviral fibroblast factor 1 (NV1FGF) or placebo and underwent a systematic ophthalmologic examination at baseline and 3, 6, or 12 months following gene therapy. Twenty-six patients assigned to the Münster subgroup received a retinal fluorescence angiography at baseline and

final examination. Among those 26 patients, four of nine with diabetes suffered from no proliferative diabetic retinopathy, and three showed non-exudative age-related macular degeneration. No retinal morphology or function change was observed in the Münster subgroup of both TALISMAN trials independent of the intramuscular NV1FGF dosage applied. Therefore, the researchers concluded that patients with diabetic retinopathy or age-related macular degeneration should not be excluded from angiogenic gene therapy trials (Prokosch et al., 2014).

Review Questions and Outcomes

The following focused review questions were developed to determine whether gene therapy is an effective diabetes treatment and diabetes-related complications. Findings from the trials were used to answer these questions, described below.

RQ1: In patients with type 1 diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes (O)?

Outcome: Twenty-four studies involved gene therapy for type 1 diabetes. Type 1 diabetes is an autoimmune disorder; therefore, current research focuses on the malfunctioning immunoregulatory pathways in type 1 diabetes patients to prevent, halt progression, or cure this disease (UCSF, 2022). Six studies focused on interleukin cells (Seelig et al., 2018; Hartemann et al., 2013; Iltoo Pharma, 2015; Marcovecchio et al., 2020; Rosenzwajg et al., 2020; Todd et al., 2016a), two on dendritic cells (Avotres Inc., 2019; Diavacs, 2015), two on proinsulin hormone (Precigen Actobio, 2018; Roep et al., 2013), two on monoclonal antibodies (Marwaha et al., 2022; ProventioBio, 2022), two on mesenchymal stem cells (Gwoxi Stem cell applied technology, 2021; National Institute of Diabetes and Digestive and Kidney Diseases, 2019), one on antigen-specificity (University of Colorado, 2022), three on T regulatory cells for glucose stabilization (Bluestone et al., 2015; Caladrius Biosciences, 2019; Dong et al., 2021), two on T

regulatory cell immunotherapy with islet transplantation (Diabetes Research Institute, 2018; University of Alberta, 2022a), and four on xenotransplantation with porcine cells in type 1 diabetes patients (Cooper et al., 2016; Hunan Xeno-life Science, 2013; Matsumoto et al., 2016; Matsumoto, Tan, Baker, Durbin, Tomiya, Azuma, Doi, et al., 2014). The four completed studies on interleukin demonstrated that interleukin cells have a dose-dependent relationship with T regulatory cells and can induce T regulatory cell function, which can, in turn, prevent the destruction of the pancreatic beta cells, thereby reducing the progression of type 1 diabetes (Hartemann et al., 2013; Rosenzwajg et al., 2020; Seelig et al., 2018; Todd et al., 2016a). One dendritic cell therapy study demonstrated successfully that genetically modified dendritic cells could control high blood sugar in patients with type 1 diabetes (Diavacs, 2015; The American Journal of Managed Care, 2014). Two proinsulin and proinsulin gene therapy trials showed efficacy in blood sugar control in people with type 1 diabetes (Precigen Actobio, 2021; Roep et al., 2013). Both studies with monoclonal antibody drugs, Teplizumab and Ustekinumab, demonstrated their efficacy in stopping the progression of type 1 diabetes in treatment groups (Herold et al., 2019; Marwaha et al., 2022). Treatments with T regulatory cells delivered mixed results; one study showed positive results in keeping C-peptide levels stable, another demonstrated negative results in keeping C-peptide levels stable, while a combination of T regulatory cells with interleukin cells caused harm in the treatment group and resulted in the termination of the trial (Bluestone et al., 2015; Caladrius Biosciences, 2019; Dong et al., 2021). All reported xenotransplantation using encapsulated porcine cells was found to be successful in type 1 diabetes patients (Cooper et al., 2016; Matsumoto et al., 2016; Matsumoto, Tan, Baker, Durbin, Tomiya, Azuma, & Elliott, 2014).

All but two studies using T regulatory cells for glucose stabilization demonstrated positive outcomes. Therefore, it could be concluded that in patients with type 1 diabetes, gene therapy using interleukin cells, dendritic cells, proinsulin hormone, monoclonal antibodies, mesenchymal stem cells, antigen-specificity, T regulatory immunotherapy with islet transplantation, xenotransplantation with porcine cells in type 1 diabetes patients shown a positive difference in managing diabetes. In fact, monoclonal antibody treatments for type 1 diabetes using Teplizumab cut down disease progression by about two years and reduced the number of new onset type1 diabetes by half in people at a high risk of getting type 1 diabetes. Teplizumab is currently being reviewed by the United States Food and Drug Administration for approval to be marketed as a disease-modifying drug for type 1 diabetes (ProventioBio, 2022).

RQ2: In patients with type 2 diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes (O)?

Outcome: Six studies focused on gene therapy for type 2 diabetes. Two of these studies used a cell-based gene therapy approach using mesenchymal stem cells (Ukraine Association of Biobank, 2020; Vinmec Research Institute of Stem Cell and Gene Technology, 2017), three used glucokinase gene therapy for type 2 diabetes (AstraZeneca, 2020), and one used p53 gene therapy (Shenzhen SiBiono GeneTech Co., 2015). The completed mesenchymal cell-based gene therapy trial was found successful in reducing HbA1c and fasting blood sugar levels in patients with type 2 diabetes duration of fewer than 10 years and a body mass index of under 23 kg/m2 but not in patients with diabetes duration over 10 years and a body mass index of 23 kg/m2 (Nguyen et al., 2021; Vinmec Research Institute of Stem Cell and Gene Technology, 2017). The p53 gene therapy in patients with hepatocellular carcinoma and type 2 diabetes reduced blood sugar levels. The two completed glucokinase activator trials demonstrated that glucokinase

activators reduce blood sugar and HbA1c levels for about 4 months but are not as effective beyond 4 months. Unfortunately, no generalized inference could be made about the efficacy of gene therapy based on the scant number of studies for type 2 diabetes that used different therapeutic pathways to manage diabetes. The only conclusions can be drawn are that gene therapy with glucokinase activators effectively manages diabetes for about 4 months, gene therapy with mesenchymal stem cells is only effective in patients with diabetes duration under 10 years, and body mass index of 23 kg/m2, and p53 gene therapy is effective in maintaining blood sugar in type 2 diabetes patients concurrent with hepatocellular carcinoma.

RQ3: In patients with diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes-related neural/nerve disorders (O)?

Outcome: Five studies were found that focused on gene therapy for diabetes-related nerve disorders or neuropathy. One trial investigated the efficacy of vascular endothelial intramuscular gene transfer (VEGF) for diabetic polyneuropathy (Ropper et al., 2009), and the other four investigated VM202, a plasmid DNA encoding two isoforms of hepatocyte growth factor (HGF). VEGF reduced sensory loss and pain in patients with diabetic polyneuropathy, but the treatment came with many serious adverse events (Ropper et al., 2009). On the other hand, all four trials with VM202 successfully decreased sensory loss and pain, especially for those not on gabapentin or pregabalin (Ajroud-Driss et al., 2013; Ajroud-Driss et al., 2015; Kessler et al., 2021; Kessler et al., 2015). Therefore, VM202 gene therapy showed a positive difference in managing diabetes-related nerve disorders in patients with diabetes, especially those not gabapentin or pregabalin.

RQ4: In patients with diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing critical limb ischemia (O)?

Outcome: Seven studies investigated gene therapy for critical limb ischemia in patients with diabetes (Barć et al., 2021; Belch et al., 2011; Cui et al., 2015; Grossman et al., 2016; Gu et al., 2019; Gu et al., 2011; Hammad et al., 2020; Kusumanto et al., 2006). One used plasmid mediated vascular endothelial growth factor, two used plasmid mediated hepatocyte growth factor, one used a fibroblast growth factor, one used a combination of vascular endothelial and hepatocyte growth factor, one used an autologous endothelial and smooth muscle cells to transfer angiogenic genes, and the last one used stromal cell-derived factor-1 plasmid treatment to reduce pain, ulceration, gangrene, and rate of amputation in patients with critical limb ischemia complicated by diabetes. Gene therapy with plasmid-mediated endothelial growth factor (Kusumanto et al., 2006), hepatocyte growth factor (Cui et al., 2015; Gu et al., 2019; Gu et al., 2011), and a combination of endothelial and hepatocyte growth factor (Barć et al., 2021) found to be successful in reducing pain, ulceration, gangrene, and rate of amputation in treatment groups without significant adverse events. Gene therapy using autologous endothelial and smooth muscle cells to transfer angiogenic genes also successfully reduced pain and increased mobility in treatment groups (Grossman et al., 2016). On the other hand, gene therapy with plasmid-mediated fibroblast growth factor did not significantly reduce the number of amputations or death in the treatment groups (Belch et al., 2011), and the stromal cell-derived factor-1 plasmid treatment failed to achieve any of the endpoints such as pain reduction, wound healing and increase in blood circulation (Hammad et al., 2020). Therefore, it can be concluded that gene therapy with plasmid-mediated endothelial growth factor, hepatocyte growth factor, and a combination of endothelial and hepatocyte growth factor autologous endothelial and

smooth muscle cells to transfer angiogenic genes shown a positive difference in patients with critical limb ischemia complicated by diabetes.

RQ5: In patients with diabetes (P), has gene therapy (I), compared to pharmaceutical agents or placebo (C), shown a difference in managing diabetes-related eye complications (O)?

Outcome: Four studies focused on diabetes-related eye complications. One of these trials investigated a gene therapy product to inhibit vascular endothelial growth factor for diabetic retinopathy and successfully improved vision in one of the treatment groups thus far. The trial is ongoing and while there is no conclusive evidence can be drawn about the product's efficacy, the interim trial results show that inhibiting vascular endothelial growth factor is an effective way of treating retinopathy (REGENXBIO, 2020, 2022). The second gene therapy trial for diabetic macular edema using an adeno-associated virus vector encoding aflibercept failed to restore vision in patients in the higher dose group but were effective in the lower dose group. However, the trial was canceled due to a high number of serious adverse events in the higher dose group (HCP Live, 2021). The third trial used a viral vector to deliver a gene that expresses a therapeutic protein in the affected eye of the patients with exudative age-related macular degeneration. The study occurred in three stages: phase 1, phase 2a, and a 3-year follow-up. All stages were found to be successful in improving vision. While the study population was too small (12 in phase I and 21 in phase II) to infer a conclusion about the efficacy of this product, the process of blocking the vascular endothelial growth factor worked (Rakoczy et al., 2019). The fourth study was a retrospective investigation proving that angiogenic gene therapy does not cause retinal pathology in patients with diabetic retinopathy or macular edema (Prokosch et al., 2014). Based on the findings in the studies that focused on diabetes-related eye complications, it can be concluded

that inhibiting vascular endothelial growth factors with gene threapy is an effective way of treating diabetic vascular eye disorders.

In this chapter, 47 ongoing and completed trials have been synthesized. Twenty-four studies focused on gene therapy for type 1 diabetes, six on type 2 diabetes, and 17 on diabetes-related complications. Fourteen out of the 16 completed trials with gene therapy for type 1 diabetes, four out of the five completed trials for type 2 diabetes, and 12 out of 15 completed studies in diabetes-related complications showed a positive outcome. This systematic review aimed to determine whether gene therapy is an effective diabetes treatment. From the high number of favorable results demonstrated by the studies, it can be concluded that gene therapy is an effective treatment for diabetes. A detailed discussion of the findings will be provided in the next chapter.

Chapter Five

Discussion

Diabetes is a chronic condition characterized by excess blood sugar in the body. This condition occurs in genetically predisposed individuals who cannot neutralize blood sugar effectively due to either insulin shortage, insulin resistance, or both (American Diabetes Association, 2021a). The excess sugar in the blood causes macro and microvascular complications such as nerve disease, vision loss, cardiovascular diseases, kidney failures, and increase morbidity and mortality in those individuals. Globally, diabetes is one of the top 10 causes of mortality and the top six causes of morbidity (International Diabetes Federation, 2020). An estimated 4.2 million deaths were caused by diabetes and related complications in 2019, meaning one person died from diabetes and related complications every eight seconds in 2019 (International Diabetes Federation, 2019b). Diabetes is one of the costliest diseases to have. The Centers for Disease Control and Prevention considers diabetes the most expensive disease in America because one in every four healthcare dollars is spent on caring for people with diabetes (Centers for Disease Control and Prevention, 2022).

With conditions like diabetes, evidence-based practice of medicine can provide the most effective care and improve patient outcomes. In the evidence-based practice of medicine, systematic reviews are placed at the top of the evidence pyramid because they select, appraise, synthesize, and summarize current research results and help the clinicians provide the best treatment based on evidence (Linares-Espinós et al., 2018; Livinski, 2015; Pollock & Berge, 2018). Thus, for this project, a systematic review of gene therapy for diabetes was conducted, which included including 47 ongoing and completed gene therapy trials. Twenty-four of those studies investigated potential gene therapy solutions based on the causal pathways for type 1 diabetes, six for type 2 diabetes, and 17 for diabetes-related complications.

Type 1 diabetes is an autoimmune disorder caused by genes involved in immune regulation, called “tolerance.” The immune pathogenesis of type 1 diabetes begins with a breakdown in self-tolerance. In a normal human body, two major classes of lymphocytes, B and T cells, are responsible for self-tolerance and immune defense. The T cells control cell-mediated immunity, and the B cells control antibody-mediated immunity. Type 1 diabetes starts with a breakdown in either or both of these immunological pathways; the body loses tolerance towards its own insulin-producing pancreatic beta cells and initiates a specific targeted immune response causing the destruction of these cells leading to a lifelong dependency on exogenous insulin (Merck & Co., 2022). Extensive efforts are being made to develop novel therapies that can stop this autoimmune destruction and preserve insulin production. Therefore, it is not surprising that 20 out of the 24 studies on type 1 diabetes focused on immunoregulation. Gene therapy with interleukin cells (Seelig et al., 2018; Hartemann et al., 2013; Iltoo Pharma, 2015; Marcovecchio et al., 2020; Rosenzwajg et al., 2020; Todd et al., 2016a), dendritic cells (Avotres Inc., 2019; Diavacs, 2015), and Treg cells (Bluestone et al., 2015; Caladrius Biosciences, 2019; Diabetes Research Institute, 2018; Dong et al., 2021; University of Alberta, 2022a) targeted T cell mediated immunity while the studies with proinsulin hormone (Precigen Actobio, 2018; Roep et al., 2013), monoclonal antibodies (Marwaha et al., 2022; ProventioBio, 2022), mesenchymal stem cells (Gwoxi Stem cell applied technology, 2021; National Institute of Diabetes and Digestive and Kidney Diseases, 2019), and antigen-specific insulin B chain (University of Colorado, 2022) targeted B cell mediated immunity.

The T regulatory (Treg) cells are a subset of T cells that regulate the T cell-mediated immune response in a human body, and therefore possess the great potential to stop the autoimmunity that causes type 1 diabetes (Merck & Co., 2022). Treg cells demonstrated their

efficacy in preclinical studies for type 1 diabetes treatment by slowing down diabetes progression and reversing new-onset diabetes in diabetic mice (Bluestone et al., 2015). Bluestone et al. at the University of San Francisco found that a single Treg infusion in study participants in four different dose cohorts kept their C-peptide levels stable for one year and in two cohorts for two years without serious adverse events (Bluestone et al., 2015). Interim results from an ongoing trial conducted by Caladrius Bioscience also demonstrated that Treg cells stabilized C-peptide levels in the treatment group without any adverse events (Caladrius Biosciences, 2019). As interleukin cells work with Treg cells to suppress excessive immune activation and prevent autoimmunity, Bluestone et al. conducted a study with Treg and interleukin-2 cells, hoping to increase treatment efficacy. Unfortunately, this combination caused the opposite of the intended effect and reduced C-peptide levels in treatment groups instead of increasing them. The trial was considered unsafe by the Data Safety Monitoring Board and eventually shut down (Dong et al., 2021).

While Bluestone et al. (2021) learned that interleukin cell and Treg cell infusion together does not stabilize C-peptide levels, Hertemann et al. (2011), Todd et al. (2016), Seelig et al. (2018), and Rosenzwajg et al. (2020) successfully demonstrated that using only interleukin-2 (IL-2) cells can stabilize C-peptide levels. The researchers also established that IL-2 has a dose-dependent relationship with Treg cells and can increase the frequency of Tregs to suppress the destruction of insulin-producing beta cells and stabilize blood glucose and HbA1c levels. Even though these studies included fewer than 50 people, the repeatability of the results in different studies confirmed the dose dependency of IL-2 and Treg cells and their ability to arrest the autoimmune destruction of beta cells and type 1 diabetes progression. Current ongoing trials are investigating Aldesleukin, an IL-2 product, for the precise IL-2 dosage necessary for an optimal

Treg induction and reduction of the autoimmune destruction of beta cells (Iltoo Pharma, 2015; Marcovecchio et al., 2020). Aldesleukin was originally approved by the U.S. Food and Drug Administration for metastatic melanoma in 1998 as one of the few medications that successfully used the body's own immune system to battle metastasized cancer (U.S. Food and Drug Administration, 2021b). Repurposing this drug with the correct dosage to prevent type 1 diabetes disease progression has great potential to reduce insulin dependence and diabetes-related complications in type 1 diabetes patients. Drug repurposing, or repositioning, is encouraged by the 21st Century Cures Act because it significantly reduces the cost and time of developing new medications, and some of the savings could be passed on to the patients (Challener, 2017).

Currently dendritic cells for type1 diabetes are being investigated by Diavacs (2015) and Avotres, Inc. (2019). Dendritic cells present antigens (foreign substances) to T cells to initiate a T-cell-mediated immune response and destroy these antigens. Preclinical trials demonstrated that people with autoimmune disorders often have faulty dendritic cells that cannot distinguish between "self" and "non-self" and mistakenly present the body's own cells as antigens to T cells to be destroyed (Avotres Inc., 2019; Diavacs, 2015). Therefore, if dendritic cells could be genetically modified to recognize "self" from "non-self," the autoimmune process would stop, and the beta-cells could be saved from destruction. Diavacs and Avotres are investigating whether genetically enhanced autologous dendritic cells can stop the destruction of pancreatic beta cells in subjects with new-onset type 1 diabetes. Diavac's phase I trial successfully demonstrated that dendritic cell therapy can stabilize glucose in type 1 diabetes patients (Diavacs, 2015). If future trials with dendritic cells continue to show success, this would be another effective way of preventing disease progression in type 1 diabetes patients.

A different technique to induce self-tolerance towards pancreatic beta cells is being explored by Precigen ActoBio and Tolerion Inc. Their trials are currently investigating whether self-tolerance towards beta cells could be induced with genetically modified proinsulin. Interim results from the trials showed that genetically modified proinsulin drugs AG019 and TOL-3021 effectively stabilized glucose, HbA1c, and C-peptide levels in treatment groups (Precigen Actobio, 2018, 2021; Tolerion, 2019). These studies' positive outcomes suggest that this method could also induce self-tolerance in type 1 diabetes patients.

The University of Colorado at Denver, in collaboration with Nova Immunotherapeutics Limited, is currently conducting an antigen-specific study for type 1 diabetes with MER3101, a drug composed of insulin B chain and MAS-1 adjuvant. The insulin B chain is part of an insulin molecule, and an adjuvant is a substance that stimulates the immune system to react strongly to an antigen (University of Colorado, 2022). Interestingly, this study design is similar to an allergy immunotherapy treatment where a patient receives incremental doses of a specific allergen or antigen to desensitize the body against this particular antigen (American College of Allergy, 2022). In this study, the insulin B chain is being used as an antigen to reduce sensitivity towards the entire insulin molecule and reduce the autoimmune destruction of insulin-producing beta cells in type 1 diabetes (University of Colorado, 2022). Antigen-specific treatments can be tailored based on an individual's particular autoimmunity and therefore has the potential to offer treatments that are truly personalized and ensure the best possible outcomes. So far, antigen-specific treatments for other autoimmune disorders, such as rheumatoid arthritis, multiple sclerosis, lupus, and celiac disease, demonstrated success in preliminary trials (Hirsch & Ponda, 2015).

The trials conducted with monoclonal antibody drugs, Teplizumab and Ustekinumab, demonstrated that monoclonal antibodies can also increase self-tolerance, leading to a reduction of beta cell destruction, delayed disease onset, and progression in type 1 diabetes patients (Herold et al., 2019; Juvenile Diabetes Research Foundation, 2015). Monoclonal antibodies (mAbs) are produced by B cells and specifically target antigens. Teplizumab is currently under review by the United States Food and Drug Administration and is anticipated to receive approval by November 17, 2022, as a first-of-its-kind, disease-modifying drug for type 1 diabetes (ProventioBio, 2022). It is worth noting that monoclonal antibody treatments targeting interleukin cells is another example of drug repositioning initially developed to treat cancer in 1986 (Lu et al., 2020). Since then, they have not only successfully treated cancer but also Crohn's disease, ulcerative colitis, plaque psoriasis, and psoriatic arthritis (Benson et al., 2011) and COVID-19 (National Institutes of Health, 2022). Repurposing monoclonal antibodies for type 1 diabetes can save money and time involved with new drug development, and cost savings could be passed onto the patients.

The Edmonton protocol, an experimental treatment for type 1 diabetes in phase III clinical trial, was discussed in the literature review (National Institutes of Health, 2021; University of Alberta, 2022b). The Edmonton protocol refers to a pancreatic islet transplantation procedure where islets with healthy beta cells are collected from the pancreas of a deceased organ donor and then injected into a vein that carries blood to the liver of a person with type 1 diabetes to produce endogenous insulin. This 20-year-long study finally finished in May 2022. All transplants participants produced enough endogenous insulin with the transplanted islets for the first five years without the need for exogenous insulin. Unfortunately, the researchers noticed that the efficacy of these islets started to fade after five years, and many transplant participants

started to require small amounts of exogenous insulin. After further investigation, the researchers discovered that the immunosuppressive medication caused graft damage and transplant patients' exogenous insulin requirement. They also realized that transplantations for type 1 could not be fully successful unless the underlying cause of type 1 diabetes, the body's intolerance towards the beta cells, is addressed, which continues even after transplantation. Preclinical trials demonstrated that Tregs could contribute to self-tolerance by preventing the initiation of unwanted immune activation and by suppressing ongoing immune responses toward graft destruction. The researchers are now conducting two trials to investigate the safety and efficacy of Treg cell infusion for transplanted islet cell preservation (Diabetes Research Institute, 2018; University of Alberta, 2022a). If these trials show positive outcomes, the islet transplant patients will be able to maintain their insulin independence beyond five years without the serious side effects of anti-rejection medications and experience higher quality of life.

Unfortunately, donor islets are scarce, and the scarcity is expected to continue. In 2017, only 31,812 organ donors were available globally for 422 million people with diabetes. Approximately 400,000 working beta cells must be transplanted into a patient to stabilize blood sugar, and often multiple donors are needed to find this many islets (National Institutes of Health, 2021). Consequently, with some failed islet transplantation and the necessity for multiple donors, the Edmonton protocol could only help 0.001% of the world's diabetic population per year (Shapiro, 2018). To bypass the donor organ shortage problem, xenotransplantation is being considered. The prefix "xeno" comes from the latin word "xénos," which means "foreign"; therefore, the term xenotransplantation refers to a procedure that involves the transplantation of live cells, tissues, or organs from a foreign source, usually a nonhuman animal source (Deschamps et al., 2005). Diatranz Otsuka Ltd. and Hunan Xeno-life Science Ltd are currently

investigating whether genetically modified porcine islet cells could be used for transplantation instead of human pancreatic islets. It was discussed in the literature review that porcine islet cells are suitable for human transplantation because they have a similar “set point” for insulin production and can be genetically modified using gene-editing technology to reduce the risk of immune rejection by the human body. After these genetically modified porcine islets successfully demonstrated their safety and efficacy in nonhuman primates, they were allowed to enter clinical trials with human subjects (Bellin & Dunn, 2020).

All three xenotransplantation trials conducted by Diatranz Otsuka Ltd. using encapsulated porcine cells successfully stabilized glucose in type 1 diabetes patients without any serious adverse events (Cooper et al., 2016; Matsumoto et al., 2016; Matsumoto, Tan, Baker, Durbin, Tomiya, Azuma, & Elliott, 2014). These trials utilized encapsulated porcine cells in alginate microcapsules called DIABECELL to avoid rejection by the body. Over the last decade, researchers made tremendous advancements with xenotransplantation to get around the donor organ shortage problem. So far, the University of Maryland at Baltimore researchers have conducted a life-sustaining heart xenotransplant in a patient with end-stage heart failure (Rothblatt, 2022); New York University Langone Health (Rabin, 2022) and the University of Alabama at Birmingham (Porrett et al., 2022) have performed kidney xenotransplants in brain-dead human subjects. Xenotransplantations with porcine cells such as DIABECELLs has the capacity to reduce insulin dependence in type 1 diabetes patients and type 2 diabetes patients since type 2 diabetes also progresses towards insulin dependency (American Diabetes Association, 2022a).

Gene therapy with mesenchymal stem cells can also potentially treat type 1 and 2 diabetes. Mesenchymal stem cells are multipotent stem cells that can generate new tissues,

suppress autoimmunity and deliver new genes; these characteristics make them invaluable for type 1 and type 2 diabetes treatment (Kim et al., 2019). Two trials using these cells for type 1 diabetes are ongoing and have not reported any interim results (Gwoxi Stem cell applied technology, 2021; National Institute of Diabetes and Digestive and Kidney Diseases, 2019). The type 2 diabetes trial that the Ukraine Association of Biobank was conducting reported no updates on the clinical trials site and has possibly been impacted by the current war in Ukraine (QPS, 2022). The completed trial for type 2 diabetes was conducted by Vinmec Research Institute (Nguyen et al., 2021). The study demonstrated that autologous bone marrow-derived mesenchymal stem cell transplantation could successfully reduce glucose and HbA1c levels and stabilize C-peptide levels without serious adverse events. The trial results also revealed that participants with diabetes duration of less than 10 years and a body mass index under 23 kg/m2 experienced a significant reduction in their HbA1c levels and a moderate reduction in fasting blood glucose levels and C-peptide levels that lasted for 12 months where the other participants with diabetes duration of more than 10 years and body mass index over 23 kg/m2 enjoyed these levels only for the first three months (Nguyen et al., 2021). This phenomenon likely implies that type 2 diabetes patients with duration less than 10 years and body mass index under 23 kg/m2 have less damage in their beta cells and less insulin resistance, and therefore these cells could be more effectively repaired with mesenchymal stem cells. Additionally, this particular finding emphasizes the importance of early intervention with gene therapy for type 2 diabetes.

One of the other studies for type 2 diabetes explored p53 gene therapy using Gendecine and successfully reduced blood sugar levels in patients with type 2 diabetes and hepatocellular carcinoma (Shenzhen SiBiono GeneTech Co., 2015). Gendicine was discussed in the literature review as the world's first-ever commercially approved gene therapy product. This drug was

originally developed to treat head and neck cancer (Ma et al., 2020). It seems that Gendicine is now being repositioned to treat liver cancer in type 2 diabetes patients. People with type 2 diabetes face a much higher risk for pancreatic, liver, colon, breast, and endometrial cancer than those without diabetes (Bjornsdottir et al., 2020; Ling et al., 2020). The 2015 trial demonstrated that Gendicine could successfully treat liver cancer and lower blood sugar in patients with type 2 diabetes (Shenzhen SiBiono GeneTech Co., 2015). Future trials could be conducted to investigate whether Gendicine can treat other cancers in people with type 2 diabetes.

Three studies considered glucokinase activators for type 2 diabetes (AstraZeneca, 2020; Ericsson et al., 2012; Kiyosue et al., 2013). Glucokinase is an enzyme encoded by the GCK genes in a normal human body that controls glucose regulation. Glucokinase activators increase glucokinase production by enhancing GCK gene expression and establishing glucose homeostasis (DiabetesGenes, 2022). The two completed glucokinase activator trials conducted by AstraZeneca demonstrated that glucokinase activators could reduce blood sugar and HbA1c levels for about 4 months, with effects tapering off beyond 4 months (AstraZeneca, 2020; Ericsson et al., 2012). While AstraZeneca needs to conduct more trials to demonstrate the efficacy of this drug and receive approval from the U.S. Food and Drug Administration to market this product, a glucokinase activator called HuaTangNing, developed by Bayer and Hua Medicine, received approval from the China Food and Drug Administration (Fiercebiotech, 2022). In the phase III trial, HuaTangNing stabilized blood sugar and HbA1c levels for up to a year in patients who failed to regulate their blood sugar with a maximum daily dose of metformin (Hua Medicine, 2020). This drug is now available for type 2 diabetes treatment outside of the United States and the European Union (Fiercebiotech, 2022).

People with any type of diabetes suffer from micro and macrovascular complications (International Diabetes Federation, 2020). Diabetic nerve damage or neuropathy is a microvascular complication. Approximately 33% to 50% of diabetes patients suffer from peripheral neuropathy that affects their feet, legs, and sometimes hands and arms. The symptoms of peripheral neuropathy start with tingling, then progress to burning, throbbing, stabbing pain, loss of sensation, muscle coordination, and balance in patients with diabetes (The National Institute of Diabetes and Digestive and Kidney Diseases, 2022). Current treatment only includes palliative care with Lyrica (pregabalin) and Neurontin (gabapentin) to lower pain. There is a dire need for more effective treatment options for people with diabetic neuropathy. Gene therapy trials are ongoing to address this need. Five studies explored gene therapy for diabetic neuropathy (Ajroud-Driss et al., 2013; Ajroud-Driss et al., 2015; Kessler et al., 2021; Kessler et al., 2015; Ropper et al., 2009).

One of these trials investigated the efficacy of vascular endothelial growth factor (VEGF) for diabetic polyneuropathy (Ropper et al., 2009), and the other four examined VM202, a plasmid DNA encoding two isoforms of hepatocyte growth factor (HGF) (Ajroud-Driss et al., 2013; Ajroud-Driss et al., 2015; Kessler et al., 2021; Kessler et al., 2015). As the name suggests, growth factors stimulate the growth of specific tissues; therefore, growth factors such as VEGF and HGF have the potential to induce angiogenesis (blood vessel formation) and neurogeneration (nerve cell formation) (National Center for Biotechnology Information, 2022). Ropper et al. (2009) discovered that VEGF successfully treated sensory loss and pain in patients with diabetic polyneuropathy but caused many serious adverse events. On the other hand, HGF (VM202) in all four trials conducted by Helixmith Co., Ltd, significantly decreased sensory loss and pain and improved mobility in patients with diabetic neuropathy, especially those not on gabapentin or

pregabalin (Ajroud-Driss et al., 2013; Ajroud-Driss et al., 2015; Kessler et al., 2021; Kessler et al., 2015). The researchers are currently conducting a second phase III trial, estimated to finish this year, to ensure U.S. FDA approval (Kessler et al., 2021). Once approved, it will be the first regenerative medicine to treat diabetic polyneuropathy and stop disease progression (Bioinformant, 2022b).

Critical limb ischemia is another microvascular complication of diabetes. People with diabetes face a 4 times higher risk of suffering from critical limb ischemia than those without diabetes (Barnes et al., 2020). It is caused by reduced blood flow to the hands, legs, and feet and characterized by constant rest pain, ulceration, and gangrene leading to amputation (Simon et al., 2022). Currently available treatments include invasive treatments such as angioplasty, atherectomy, and revascularization surgery (Barnes et al., 2020). Kusumanto et al. (2006) used plasmid-mediated endothelial growth factor, Gu et. al. (2011), Cui et al. (2015), and Gu et al. (2019) utilized hepatocyte growth factor, and Barć et al. (2021) employed a combination of endothelial and hepatocyte growth factors to treat critical limb ischemia in diabetic patients. They found that these growth factors can successfully induce angiogenesis in patients with critical limb ischemia and significantly reduce pain, ulceration, gangrene, and the rate of amputation without any significant adverse events.

On the other hand, Belch et al. (2011) failed to significantly reduce the number of amputations or death in the treatment groups with plasmid-mediated fibroblast growth factor. The stromal cell-derived factor-1 plasmid treatment also failed to reduce pain, wound healing, and increase blood circulation (Hammad et al., 2020). However, gene therapy using autologous endothelial and smooth muscle cells to transfer angiogenic genes successfully reduced pain and increased mobility in treatment groups (Grossman et al., 2016). A gene therapy product with

hepatocyte growth factor (HGF), called Collategene, developed by AnGes Inc., is currently being used in clinical settings to treat critical limb ischemia in Japan after receiving conditional approval from the Japanese Regulatory Authority In 2019 (Ylä-Herttuala, 2019).

Diabetes increases the risk for vascular eye complications such as retinopathy, macular edema, and age-related macular degeneration that cause blindness. According to the National Eye Institute (2022), diabetic retinopathy affects more than 50% of the patients with diabetes, and diabetic macular edema affects 1 in 15 people with diabetes. The presence of diabetes also increases the rate and decreases the onset time for age-related macular degeneration (National Eye Institute, 2022b). All three eye complications happen due to abnormal neovascularization (growth of new blood vessels), a response to vascular endothelial growth factor released by the hypoxic (deprived of oxygen) retina of a person with diabetes (Kellogg Eye Center, n.d.). Current therapies for these diseases include laser treatment and frequent ocular injections of anti-vascular endothelial growth factor drugs. Unfortunately, these painful and expensive treatments lead to undertreatment, disease progression, and subsequent vision loss in patients (National Eye Institute, 2022b). A more permanent solution for these complications is being pursued through gene therapy. Unfortunately, a gene therapy trial for diabetic macular edema using an adeno-associated virus vector encoding aflibercept intended to reduce treatment frequency failed to restore vision in patients. The trial was canceled due to many serious adverse events (HCP Live, 2021). REGENXBIO, Inc. is currently investigating RGX-314, a gene therapy product that includes the NAV-AAV8 vector containing a gene encoding for a monoclonal antibody fragment designed to inhibit vascular endothelial growth factor and treat diabetic retinopathy; interim results from this trial revealed that 47% of the patients in the treatment group experienced a significant improvement in their vision (REGENXBIO, 2020). Adverum Biotechnologies, Inc.

conducted a trial with rAAV.sFLT-1, a viral vector used to deliver a gene that expresses a therapeutic protein in the eye- in patients with exudative age-related macular degeneration and prevents neovascularization. The study occurred in three stages: phase 1, 2a, and a 3-year follow-up. All stages were found to be successful in improving vision (Rakoczy et al., 2019).

Most of the studies for this systematic review demonstrated their efficacy in treating type 1 and type 2 diabetes and diabetes-related complications. For type 1 diabetes, gene therapy trials to induce self-tolerance via Treg cells (Bluestone et al., 2015; Caladrius Biosciences, 2019), interleukin cells (Hartemann et al., 2013; Rosenzwajg et al., 2020; Seelig et al., 2018; Todd et al., 2016a), dendritic cells (Diavacs, 2015), genetically modified proinsulin (Precigen Actobio, 2021; Roep et al., 2013), and monoclonal antibodies (Herold et al., 2019; Marwaha et al., 2022) have been particularly successful. For type 2 diabetes, gene therapy with glucokinase activators to increase insulin production (AstraZeneca, 2020) and mesenchymal cell therapy to repair damaged beta cells also demonstrated success (Nguyen et al., 2021; Vinmec Research Institute of Stem Cell and Gene Technology, 2017). Gene therapy with different types of growth factors to induce angiogenesis successfully treated diabetic neuropathy (Ajroud-Driss et al., 2013; Ajroud-Driss et al., 2015; Kessler et al., 2021; Kessler et al., 2015) and critical limb ischemia (Barć et al., 2021; Belch et al., 2011; Cui et al., 2015; Grossman et al., 2016; Gu et al., 2019; Gu et al., 2011; Hammad et al., 2020; Kusumanto et al., 2006) and gene therapy to inhibit vascular endothelial growth factors worked to treat vascular eye disorders associated with diabetes (Rakoczy et al., 2018; Rakoczy et al., 2017; REGENXBIO, 2022). Overall, the gene therapy studies in this systematic review provided evidence that they can effectively treat type 1 and 2 diabetes diabetes-related complications.

Conclusions

Before conducting this systematic review, an extensive literature search was conducted through nine databases to find previous systematic reviews on gene therapy for diabetes in humans. Thirteen systematic reviews were discovered, where 12 focused on wide-ranging interventions for diabetes in humans: pharmaceutical agents, alternative medicine, supplementations, gum disease treatment, peer support, equity, active video gaming, and blood sugar management after stroke (Cao et al., 2021; Costello et al., 2016; Engebretson & Kocher, 2013; Giugliano et al., 2011; Höchsmann et al., 2016; Kalafat et al., 2018; Laird & Coates, 2013; Mousa et al., 2018; Patil et al., 2018; Suksomboon et al., 2011; Terens et al., 2018; Yu et al., 2018) and one on gene therapy in rodents (Ghiasi et al., 2020). However, none of the 13 systematic reviews focused on gene therapy for diabetes in humans, suggesting a significant gap in the literature on gene therapy for diabetes in humans. This project addressed this particular gap in the literature by conducting a systematic review with 47 clinical trials on gene therapy for diabetes in humans.

An additional purpose of this systematic review was to determine if gene therapy is an effective treatment for diabetes. Forty-seven ongoing and completed trials have been synthesized for this systematic review that, included 24 studies on gene therapy for type 1 diabetes, six on type 2 diabetes, and 17 on diabetes-related complications. Fourteen out of the 16 completed trials with gene therapy for type 1 diabetes, four out of the five completed trials for type 2 diabetes, and 12 out of 15 completed studies in diabetes-related complications yielded positive results. The high number of favorable outcomes demonstrated by the trials implies that gene therapy is an effective treatment for diabetes.

Limitations of the Systematic Review

The United States Food and Drug Administration offers two definitions for gene therapy: a narrow definition that states that "Gene therapy is a technique that modifies a person's genes to treat or cure disease" and a broad definition that "Human gene therapy seeks to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use" (U.S. Food and Drug Administration, 2018). For this systematic review, the broad definition of gene therapy was used to find the maximum number of studies, such as immunotherapies for diabetes, where the biological properties of specific cells were genetically modified to create immune responses in patients without causing permanent changes in their genes. Additionally, most of the studies synthesized in this systematic review were in clinical trial phases I or II, thus included fewer than 100 participants and used placebos for comparisons. Therefore, until phase III trials are completed with more participants and current pharmaceutical treatments as comparisons, the efficacy of these gene therapy products cannot be generalized to all patients with diabetes or be compared to current pharmaceutical agents used to treat diabetes or diabetes-related complications.

Recommendations for Future Research

The literature review of this project discussed the two main barriers to gene therapy: regulations and cost. Current regulations in the United States only allow somatic cell gene therapy, and even somatic cell gene therapy has additional restrictions on embryonic stem cell usage (American Society of Gene and Cell Therapy, 2020; National Academies of Sciences & Medicine, 2017). To bypass this, induced pluripotent stem cells (iPSC) could be used in gene therapy. These cells are created by harvesting mature skin or blood cells and reprogramming them into a similar pluripotent state like embryonic stem cells. The gene-editing tool CRISPR could also be used to modify these cells to any intended cell type such as beta cells for diabetes

or neurons for neurological disorders (Ben Jehuda et al., 2018). Currently, ViaCyte and CRISPR Therapeutics are conducting research to investigate the efficacy of beta cells that has been created with iPSCs and CRISPR in type 1 diabetes patients (diaTribe, 2022). Many gene therapy trials for different diseases such as cancer, eye, heart, and Parkinson's disease are also using iPSCs. The success of these trials will demonstrate the efficacy of iPSCs, reduce the need for embryonic stem cell use in gene therapy, and encourage more gene therapy research in America (Bioinformant, 2022a).

The estimated cost of research and development of a new gene therapy drug including all phases of clinical trial costs and 15–20-year follow-up ranges from $161 million to $2 billion (Kassir et al., 2020). The development cost of gene therapy could be reduced through drug repositioning or repurposing. Drug repositioning refers to the process of finding new uses for existing drugs. This process circumvents the development time and cost of drug discovery and decreases the overall cost of bringing the drug to market because the safety profiles of these existing drugs have already been established (Challener, 2017). Studies found for this systematic review revealed that existing metastatic melanoma drug Aldesleukin (Iltoo Pharma, 2015; Marcovecchio et al., 2020), monoclonal antibody therapy drugs Teplizumab and Ustekinumab (Herold et al., 2019; Marwaha et al., 2022) are being repositioned to treat type 1 diabetes while Gendicine, a head and neck cancer drug (Zhang et al., 2018), is being repositioned to treat liver cancer in people with type 2 diabetes. Finding alternate use for existing gene therapy drugs will bring down the overall cost of gene therapy.

Using in silico computational models would be another way to reduce the cost and the time usually associated with developing new gene therapy drugs. In silico models refer to predictive computer models that can test hypotheses about a drug's efficacy using the drug's

pharmacokinetic data and the pathogenesis and pathophysiology of a particular disease. These models can also generate predictions about a drug's side effects, leading to better drug design and reducing drug development costs (Piñero et al., 2018). Additionally, in-silico approaches could link genes and diseases and predict novel therapeutic targets for genetic disorders like diabetes while reducing development time and cost (Ferrero et al., 2017; Teng et al., 2021).

Gene therapy products tend to cost more because private corporations often fund them to make a profit. Kassir et al. (2020) investigated the funding of all active gene therapy trials until January 2019 and discovered that mainly private industries have been carrying the cost of this kind of drug development. Most of the studies for this systematic review were sponsored by private biotech firms and confirmed Kassir et al.'s reporting. Increasing government funding for genetic research will increase competition and bring down the cost. Only six gene therapy studies were found for type 2 diabetes in 10 databases from 2000 to 2022. Since 90% of the people with diabetes have type 2 diabetes and the number of adults with diabetes is projected to go up to 578 million by 2030 and 700 million by 2045 (International Diabetes Federation, 2019a), it is evident that current pharmaceutical agents or advice about lifestyle changes failed to solve this global crisis. It is time to acknowledge that diabetes is a genetic problem and find a genetic solution. The cost of diabetes care is projected to increase to $825 billion by 2030, and $845 billion by 2045 (International Diabetes Federation, 2019b, 2020; Lin et al., 2020; Williams et al., 2020). The responsibility of bearing much of the cost will fall onto governments worldwide. Therefore, governments need to recognize the need for better solutions than current methods available to treat diabetes and increase funding for studies in gene therapy now to decrease the cost of care in the future.

Recommendations for Clinician Education and Clinical Practice

Clinicians should be educated on the genetic and demographic aspects of diabetes to offer better screening and early care. Genetic predisposition is essential in developing type 1 or 2, the two most common forms of diabetes. The risk of getting type 1 diabetes is 30-70% for identical twins, 6-7% for siblings, and 1-9% with a parent with type 1 diabetes (DiMeglio et al., 2018; Matharoo et al., 2017; Redondo et al., 2018). For type 2, the risk is 41-55% for identical twins, 24% with a sibling, 40% with one parent, and 70% with both parents with type 2 diabetes (American Diabetes Association, 2021b; DiMeglio et al., 2018; Matharoo et al., 2017; Redondo et al., 2018). Type 1 diabetes is usually diagnosed in children and young adults, often called juvenile diabetes, and type 2 diabetes is usually diagnosed in adults, described as adult onset diabetes (Cleveland Clinic, 2022). Additionally, type 1 diabetes is more common in the non-Hispanic White population, whereas type 2 diabetes is more prevalent in African Americans, Hispanics/Latinos, American Indians, Asian Americans, and Pacific Islanders than non-Hispanic Whites (Centers for Disease Control and Prevention, 2020).

Clinicians also need to recognize that while higher body weight increases the risk for any ailment, including diabetes, higher body weight does not necessarily cause diabetes. Most people with type 1 diabetes have normal body weight at the time of diagnosis (UCSF, 2022). Recent research conducted by Zhu et al. that included a large cohort of 4.9 million adults that was racially/ethnically, geographically, and socioeconomically diverse found high susceptibility for type 2 diabetes in normal-weight (BMI>25 kg/m^2) non-White individuals (Zhu et al., 2019). Therefore, clinicians need to be educated on the current evidence that acknowledges genetic and demographic aspects of diabetes over body mass index and implement evidence-based medicine in their clinical practices accordingly. People at high risk for type 1 diabetes can be tested for antibodies directed against beta cell antigens (American Diabetes Association, 2021a; UCSF,

2022). Teplizumab is an antibody gene therapy found to delay the progression of type 1 diabetes in high-risk participants by about 2 years and cut the number of onsets by half in clinical trials (Herold et al., 2019), currently under review by the United States Food and Drug Administration and is expected to receive approval by November 17, 2022 (ProventioBio, 2022). If approved, clinicians can prescribe this drug to people at high risk for type 1 diabetes, reduce the number of onsets, and delay progression. Currently, no widely accepted genetic testing is available to measure the risk for type 2 diabetes. Researchers are working with 23andme, a genetic testing company for ancestry and traits, to develop effective predictive genetic scores based on the genetic profiles of individuals with family members who have type 2 diabetes (Ashenhurst et al., 2022). These scores may not be available for clinical practice for a while. In the meantime, clinicians can arm themselves with knowledge of current evidence that point to the genetic and demographic aspects of type 2 diabetes to identify high-risk patents and offer counseling, and treatment.

References

Adverum Biotechnologies, Inc. (2020). *ADVM-022 Intravitreal gene therapy for DME (INFINITY)*. https://clinicaltrials.gov/ct2/show/NCT04418427

Ajroud-Driss, S., Christiansen, M., Allen, J. A., & Kessler, J. A. (2013). Phase 1/2 open-label dose-escalation study of plasmid DNA expressing two isoforms of hepatocyte growth factor in patients with painful diabetic peripheral neuropathy. *Molecular Therapy*, *21*(6), 1279-1286.

Ajroud-Driss, S., Vinik, A. I., Smith, A. G., Cha, B. S., Choi, S. H., Wymer, J. P., Shaibani, A., & Kessler, J. A. (2015). Double-blind, placebo-controlled study of hepatocyte growth factor gene therapy in painful diabetic neuropathy [Conference Abstract]. *Journal of the Peripheral Nervous System*, *20*(2), 94.

American College of Allergy. (2022). *Allergy immunotherapy*. https://acaai.org/allergies/management-treatment/allergy-immunotherapy/

American Diabetes Association. (2019a). Classification and diagnosis of diabetes: Standards of medical care in diabetes—2019. *Diabetes Care*, *42*(Supplement 1), S13-S28.

American Diabetes Association. (2019b). Standards of medical care in diabetes—2019. *Diabetes Care*, *42*(Supplement 1), 172.

American Diabetes Association. (2021a). *Genetics of diabetes*. https://www.diabetes.org/diabetes/genetics-diabetes

American Diabetes Association. (2021b). *Learn the genetics of diabetes*. https://www.diabetes.org/diabetes/genetics-diabetes

American Diabetes Association. (2022a). *How type 2 diabetes progresses*. https://diabetes.org/diabetes/type-2/how-type-2-diabetes-progresses

American Diabetes Association. (2022b). *Oral medication*. https://www.diabetes.org/healthy-living/medication-treatments/oral-medication/what-are-my-options

American Society of Gene and Cell Therapy. (2020). *Ethical issues: Germline gene editing*. https://patienteducation.asgct.org/patient-journey/ethical-issues-germline-gene-editing

American Society of Gene and Cell Therapy. (2021a). *History of gene therapy*. https://asgct.org/about/history

American Society of Gene and Cell Therapy. (2021b). *Patient education - Gene therapy basics.* https://patienteducation.asgct.org/gene-therapy-101/gene-therapy-basics

Anderson, S. L., Beutel, T. R., & Trujillo, J. M. (2020). Oral semaglutide in type 2 diabetes. *Journal of Diabetes and its Complications*, *34*(4), 107520.

Ashenhurst, J. R., Sazonova, O. V., Svrchek, O., Detweiler, S., Kita, R., Babalola, L., McIntyre, M., Aslibekyan, S., Fontanillas, P., & Shringarpure, S. (2022). A polygenic score for type 2 diabetes improves risk stratification beyond current clinical screening factors in an ancestrally diverse sample. *Frontiers in Genetics*, 921.

AstraZeneca. (2020). *AZD1656 in transplantation with diabetes to promote immune tolerance (ADOPTION)*. https://clinicaltrials.gov/show/NCT05216172

Avotres Inc. (2019). *Safety, tolerability, and potential efficacy of AVT001 in patients with Type 1 diabetes*. https://clinicaltrials.gov/ct2/show/NCT03895996

Baeshen, N., Baeshen, M., Sheikh, A., Bora, R., Ahmed, M., Ramadan, H., Saini, K., & Redwan, E. (2014). Cell factories for insulin production. *Microbial Cell Factories, 13,* 141.

Balboa, D., Saarimäki-Vire, J., Borshagovski, D., Survila, M., Lindholm, P., Galli, E., Eurola, S., Ustinov, J., Grym, H., & Huopio, H. (2018). Insulin mutations impair beta-cell development in a patient-derived iPSC model of neonatal diabetes. *Elife*, *7*, e38519.

Barć, P., Antkiewicz, M., Śliwa, B., Frączkowska, K., Guziński, M., Dawiskiba, T., Małodobra-Mazur, M., Witkiewicz, W., Kupczyńska, D., & Strzelec, B. (2021). Double VEGF/HGF gene therapy in critical limb ischemia complicated by diabetes mellitus. *Journal of Cardiovascular Translational Research*, *14*(3), 409-415.

Barnes, J. A., Eid, M. A., Creager, M. A., & Goodney, P. P. (2020). Epidemiology and risk of amputation in patients with diabetes mellitus and peripheral artery disease. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *40*(8), 1808-1817.

Belch, J., Hiatt, W. R., Baumgartner, I., Driver, I. V., Nikol, S., Norgren, L., & Van Belle, E. (2011). Effect of fibroblast growth factor NV1FGF on amputation and death: a randomised placebo-controlled trial of gene therapy in critical limb ischaemia. *Lancet*, *377*(9781), 1929-1937. https://doi.org/10.1016/s0140-6736(11)60394-2

Bellin, M. D., & Dunn, T. B. (2020). Transplant strategies for type 1 diabetes: Whole pancreas, islet and porcine beta cell therapies. *Diabetologia*, *63*(10), 2049-2056.

Ben Jehuda, R., Shemer, Y., & Binah, O. (2018). Genome editing in induced pluripotent stem cells using CRISPR/Cas9. *Stem Cell Reviews and Reports*, *14*(3), 323-336.

Benson, J. M., Peritt, D., Scallon, B. J., Heavner, G. A., Shealy, D. J., Giles-Komar, J. M., & Mascelli, M. A. (2011). Discovery and mechanism of ustekinumab: A human monoclonal antibody targeting interleukin-12 and interleukin-23 for treatment of immune-mediated disorders. *mAbs*, *3*(6), 535-545.

Berger, V., & Alperson, S. (2009). A general framework for the evaluation of clinical trial quality. *Reviews on Recent Clinical Trials*, *4*(2), 79-88.

Bioinformant. (2022a). *iPSC-derived cell therapeutics in 2022*. https://bioinformant.com/ipsc-derived-cell-therapeutics/

Bioinformant. (2022b). *RMAT designations*. https://bioinformant.com/rmat/

Bjornsdottir, H. H., Rawshani, A., Rawshani, A., Franzén, S., Svensson, A.-M., Sattar, N., & Gudbjörnsdottir, S. (2020). A national observation study of cancer incidence and mortality risks in type 2 diabetes compared to the background population over time. *Scientific Reports*, *10*(1), 1-12.

Blotsky, A. L., Rahme, E., Dahhou, M., Nakhla, M., & Dasgupta, K. (2019). Gestational diabetes associated with incident diabetes in childhood and youth: A retrospective cohort study. *Canadian Medical Association Journal*, *191*(15), E410-E417.

Bluestone, J. A., Buckner, J. H., Fitch, M., Gitelman, S. E., Gupta, S., Hellerstein, M. K., Herold, K. C., Lares, A., Lee, M. R., & Li, K. (2015). Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Science Translational Medicine*, *7*(315), 315ra189-315ra189.

Bommer, C., Heesemann, E., Sagalova, V., Manne-Goehler, J., Atun, R., Bärnighausen, T., & Vollmer, S. (2017). The global economic burden of diabetes in adults aged 20–79 years: A cost-of-illness study. *The Lancet Diabetes & Endocrinology*, *5(6), 423-430.*

Bramer, W. M., Rethlefsen, M. L., Kleijnen, J., & Franco, O. H. (2017). Optimal database combinations for literature searches in systematic reviews: A prospective exploratory study. *Systematic Reviews*. *6*(1), 1-12.

Caladrius Biosciences, I. (2019). *Caladrius Biosciences reports top-line data for the phase 2a Sanford Project: T-Rex Trial of CLBS03 for recent onset type 1 diabetes*. https://www.globenewswire.com/news-release/2019/02/13/1725052/0/en/Caladrius-Biosciences-Reports-Top-Line-Data-for-the-Phase-2a-Sanford-Project-T-Rex-Trial-of-CLBS03-for-Recent-Onset-Type-1-Diabetes.html

California Institute for Regenerative Medicine. (2016). *Safety and efficacy of CLBS03 in adolescents with recent-onset type 1 diabetes (the Sanford Project T-Rex study)*. https://clinicaltrials.gov/ct2/show/NCT02691247

Cao, H., Liu, Y., Tian, Z., Lian, Y., Jia, J., Liu, M., & Li, D. (2021). Sodium-glucose cotransporter 2 inhibitors benefit to kidney and cardiovascular outcomes for patients with type 2 diabetes mellitus and chronic kidney disease: A systematic review and meta-analysis of randomized clinical trials. *Diabetes Research & Clinical Practice, 180*.

Centers for Disease Control and Prevention. (2020). *National diabetes statistics report. 2020*. https://www.cdc.gov/diabetes/library/features/diabetes-stat-report.html

Centers for Disease Control and Prevention. (2022). *Cost-effectiveness of diabetes interventions*. https://www.cdc.gov/chronicdisease/programs-impact/pop/diabetes.htm#:~:text=Diabetes%20is%20the%20most%20expensive%20chronic%20condition%20in%20our%20nation.&text=%241%20out%20of%20every%20%244,caring%20for%20people%20with%20diabetes.

Challener, C. (2017). Expediting the discovery and development of drugs. *Pharmaceutical Technology*, *41*(9), 24-25.

Chatterjee, S., Khunti, K., & Davies, M. J. (2017). Type 2 diabetes. *The Lancet*, *389*(10085), 2239-2251.

Chen, Y., & Chen, G. (2019). New genetic characteristics of latent autoimmune diabetes in adults (LADA). *Annals of Translational Medicine, 7(5).*

Cleveland Clinic. (2022). *Diabetes: An overview*. https://my.clevelandclinic.org/health/diseases/7104-diabetes-mellitus-an-overview

ClinicalTrials.gov. (2021a). *A clinical trial of dantrolene sodium in pediatric and adult patients with Wolfram syndrome*. https://clinicaltrials.gov/ct2/show/NCT02829268

ClinicalTrials.gov. (2021b). *Encapsulated cells*. https://clinicaltrials.gov/ct2/results?cond=Diabetes+Mellitus&term=encapsulated+cells+&cntry=&state=&city=&dist=&Search=Search

Colorado State University. (n.d.). *Structure of insulin*. http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/pancreas/insulin_struct.html

Constable, I. J., Pierce, C. M., Lai, C.-M., Magno, A. L., Degli-Esposti, M. A., French, M. A., McAllister, I. L., Butler, S., Barone, S. B., & Schwartz, S. D. (2016). Phase 2a randomized clinical trial: Safety and post hoc analysis of subretinal rAAV. sFLT-1 for wet age-related macular degeneration. *EBioMedicine*, *14*, 168-175.

Cooper, D. K., Matsumoto, S., Abalovich, A., Itoh, T., Mourad, N. I., Gianello, P. R., Wolf, E., & Cozzi, E. (2016). Progress in clinical encapsulated islet xenotransplantation. *Transplantation*, *100*(11), 2301.

Costello, R. B., Dwyer, J. T., & Bailey, R. L. (2016). Chromium supplements for glycemic control in type 2 diabetes: Limited evidence of effectiveness. *Nutrition Reviews*, *74*(7), 455-468.

Cring, M. R., & Sheffield, V. C. (2020). Gene therapy and gene correction: Targets, progress, and challenges for treating human diseases. *Gene Therapy*, 1-10.

Cui, S., Guo, L., Li, X., Gu, Y., Fu, J., Dong, L., Song, H., Chen, X., Lu, Y., & Hu, C. (2015). Clinical safety and preliminary efficacy of plasmid pUDK-HGF expressing human hepatocyte growth factor (HGF) in patients with critical limb ischemia. *European Journal of Vascular and Endovascular Surgery*, *50*(4), 494-501.

De Klerk, E., & Hebrok, M. (2021). Stem cell-based clinical trials for diabetes mellitus. *Frontiers in Endocrinology*, *12*.

Deschamps, J. Y., Roux, F. A., Saï, P., & Gouin, E. (2005). History of xenotransplantation. *Xenotransplantation*, *12*(2), 91-109.

Diabetes Research Institute. (2018). *PolyTreg immunotherapy in islet transplantation*. https://clinicaltrials.gov/show/NCT03444064

DiabetesGenes. (2022). *What is glucokinase (GCK)?* https://www.diabetesgenes.org/what-is-mody/what-is-glucokinase-gck/#:~:text=Glucokinase%20(GCK)%20is%20a%20gene,of%20insulin%20produced%20also%20increases.

diaTribe. (2022). *ViaCyte and CRISPR introduce new stem cell therapy for type 1 diabetes*. https://diatribe.org/viacyte-and-crispr-introduce-new-stem-cell-therapy-for-type-1-diabetes

Diavacs, I. (2015). *Autologous immunoregulatory dendritic cells for type 1 diabetes therapy*. https://clinicaltrials.gov/ct2/show/NCT02354911

DiMeglio, L. A., Evans-Molina, C., & Oram, R. A. (2018). Type 1 diabetes. *The Lancet*, *391*(10138), 2449-2462.

Dong, S., Hiam-Galvez, K. J., Mowery, C. T., Herold, K. C., Gitelman, S. E., Esensten, J. H., Liu, W., Lares, A. P., Leinbach, A. S., & Lee, M. (2021). The effect of low-dose IL-2 and Treg adoptive cell therapy in patients with type 1 diabetes. *JCI Insight*, *6*(18).

Eden, J., Levit, L., Berg, A., & Morton, S. (2011). Finding what works in health care: Standards for systematic reviews. *Publication: National Academies Press*.

Engebretson, S., & Kocher, T. (2013). Evidence that periodontal treatment improves diabetes outcomes: A systematic review and meta-analysis. *Journal of Clinical Periodontology*, *40*, 153-163.

Ericsson, H., Sjöberg, F., Heijer, M., Dorani, H., Johansson, P., Wollbratt, M., & Norjavaara, E. (2012). The glucokinase activator AZD6370 decreases fasting and postprandial glucose in type 2 diabetes mellitus patients with effects influenced by dosing regimen and food. *Diabetes Research and Clinical Practice*, *98*(3), 436-444.

Eriksen, M. B., & Frandsen, T. F. (2018). The impact of patient, intervention, comparison, outcome (PICO) as a search strategy tool on literature search quality: A systematic review. *Journal of the Medical Library Association*, *106*(4), 420.

Ferrero, E., Dunham, I., & Sanseau, P. (2017). In silico prediction of novel therapeutic targets using gene–disease association data. *Journal of Translational Medicine*, *15*(1), 1-16.

Fiercebiotech. (2022). *Bayer, Hua Medicine snag first-in-class nod in China for Roche castoff diabetes drug, but commercial value uncertain*. https://www.fiercepharma.com/pharma/bayer-hua-medicine-snag-first-class-nod-china-roche-castoff-diabetes-drug

Fineberg, S., Galloway, J., Fineberg, N., Rathbun, M., & Hufferd, S. (1983). Immunogenicity of recombinant DNA human insulin. *Diabetologia*, *25*(6), 465-469.

Gandica, R. G., Chung, W. K., Deng, L., Goland, R., & Gallagher, M. P. (2015). Identifying monogenic diabetes in a pediatric cohort with presumed type 1 diabetes. *Pediatric Diabetes*, *16*(3), 227-233.

Ghiasi, M. R., Mohammadi, H., Symonds, M. E., Tabei, S. M. B., Salehi, A. R., Jafarpour, S., Barough, L. N., Rahimi, E., Amirkhani, Z., Miraghajani, M., & Miraghajani, M. (2020). Efficacy of insulin targeted gene therapy for type 1 diabetes mellitus: A systematic review and meta-analysis of rodent studies. *Iranian Journal of Basic Medical Sciences*, *23*(4), 406-415.

Ginn, S. L., Amaya, A. K., Alexander, I. E., Edelstein, M., & Abedi, M. R. (2018). Gene therapy clinical trials worldwide to 2017: An update. *The Journal of Gene Medicine, 20(*5), e3015.

Giugliano, D., Maiorino, M. I., Bellastella, G., Chiodini, P., & Esposito, K. (2011). Treatment regimens with insulin analogues and haemoglobin A1c target of <7% in type 2 diabetes: A systematic review. *Diabetes Research & Clinical Practice*, *92*(1), 1-10.

Gorman, G. S., Chinnery, P. F., DiMauro, S., Hirano, M., Koga, Y., McFarland, R., Suomalainen, A., Thorburn, D. R., Zeviani, M., & Turnbull, D. M. (2016). Mitochondrial diseases. *Nature Reviews Disease Primers, 2*(1), 1-22.

Grossman, P. M., Mohler III, E. R., Roessler, B. J., Wilensky, R. L., Levine, B. L., Woo, E. Y., Upchurch Jr, G. R., Schneiderman, J., Koren, B., & Hutoran, M. (2016). Phase I study of multi-gene cell therapy in patients with peripheral artery disease. *Vascular Medicine*, *21*(1), 21-32.

Gu, Y., Cui, S., Wang, Q., Liu, C., Jin, B., Guo, W., Liu, C., Chu, T., Shu, C., & Zhang, F. (2019). A randomized, double-blind, placebo-controlled phase II study of hepatocyte growth factor in the treatment of critical limb ischemia. *Molecular Therapy*, *27*(12), 2158-2165.

Gu, Y., Zhang, J., Guo, L., Cui, S., Li, X., Ding, D., Kim, J. M., Ho, S. H., Hahn, W., & Kim, S. (2011). A phase I clinical study of naked DNA expressing two isoforms of hepatocyte growth factor to treat patients with critical limb ischemia. *The Journal of Gene Medicine*. *11*(13), 602-610.

Guney, M. A., Lorberbaum, D. S., & Sussel, L. (2020). Pancreatic β cell regeneration: To β or not to β. *Current Opinion in Physiology, 14*, 13-20.

Gusenbauer, M., & Haddaway, N. R. (2020). Which academic search systems are suitable for systematic reviews or meta-analyses? Evaluating retrieval qualities of Google Scholar, PubMed, and 26 other resources. *Research Synthesis Methods, 11*(2), 181-217.

Gwoxi Stem cell applied technology, C. (2021). *Evaluate the safety of adipose-derived mesenchymal stem cell transplantation for type 1 diabetes treatment.* https://clinicaltrials.gov/ct2/show/NCT05308836

Haddaway, N., McGuinness, L., & Pritchard, C. (2021). *PRISMA2020: R package and ShinyApp for producing PRISMA 2020 compliant flow diagrams (Version 0.0. 2)*. https://estech.shinyapps.io/prisma_flowdiagram/

Haddaway, N., Woodcock, P., Macura, B., & Collins, A. (2015). Making literature reviews more reliable through application of lessons from systematic reviews. *Conservation Biology*, *29*(6), 1596-1605.

Hammad, T. A., Rundback, J., Bunte, M., Miller, L., Patel, P. D., Sadanandan, S., Fitzgerald, M., Pastore, J., Kashyap, V., & Henry, T. D. (2020). Stromal cell–derived factor-1 plasmid treatment for patients with peripheral artery disease (stop-pad) trial: Six-month results. *Journal of Endovascular Therapy, 27*(4), 669-675.

Hartemann, A., Bensimon, G., Payan, C. A., Jacqueminet, S., Bourron, O., Nicolas, N., Fonfrede, M., Rosenzwajg, M., Bernard, C., & Klatzmann, D. (2013). Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial. *The Lancet Diabetes & Endocrinology*, *1*(4), 295-305.

Harvard University. (2021). *Mitochondrial transfer: The making of three-parent babies*. https://sitn.hms.harvard.edu/flash/2018/mitochondrial-transfer-making-three-parent-babies/

HCP Live. (2021). *Gene therapy ADVM-022 provides mixed efficacy, safety in treating DME*. https://www.hcplive.com/view/gene-therapy-advm-022-mixed-efficacy-safety-treating-dme

Herold, K. C., Bundy, B. N., Long, S. A., Bluestone, J. A., DiMeglio, L. A., Dufort, M. J., Gitelman, S. E., Gottlieb, P. A., Krischer, J. P., & Linsley, P. S. (2019). An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. *New England Journal of Medicine*, *381*(7), 603-613.

Higgins, J. P., Thomas, J., Chandler, J., Cumpston, M., Li, T., Page, M. J., & Welch, V. A. (2019). *Cochrane handbook for systematic reviews of interventions*. John Wiley & Sons.

Hirsch, D. L., & Ponda, P. (2015). Antigen-based immunotherapy for autoimmune disease: current status. *ImmunoTargets and Therapy*, *4*, 1.

Höchsmann, C., Schüpbach, M., & Schmidt-Trucksäss, A. (2016). Effects of exergaming on physical activity in overweight individuals. *Sports Medicine*, *46*(6), 845-860.

Honzawa, N., & Fujimoto, K. (2021). The plasticity of pancreatic β-Cells. *Metabolites*, *11*(4), 218.

Hua Medicine. (2020). *Hua Medicine successfully completes its registration phase III trials*. https://www.huamedicine.com/En/news-203

Hunan Xeno-life Science, Ltd. (2013). *Safety and efficacy study of islets xenotransplantation.* https://clinicaltrials.gov/show/NCT03162237

Iltoo Pharma. (2015). *Low-dose rhil-2 in patients with recently-diagnosed type 1 diabetes (DIABIL-2)*. https://clinicaltrials.gov/ct2/show/NCT02411253

International Diabetes Federation. (2018). *Access to medicines and supplies for people with diabetes*. https://diabetesatlas.org/upload/resources/previous/files/8/IDF_DA_8e-EN-final.pdf

International Diabetes Federation. (2019a). *IDF diabetes atlas*. https://diabetesatlas.org/en/sections/individual-social-and-economic-impact.html

International Diabetes Federation. (2019b). *Worldwide toll of diabetes*. https://www.diabetesatlas.org/en/sections/worldwide-toll-of-diabetes.html

International Diabetes Federation. (2020). *Diabetes facts & figures*. https://idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html

Juvenile Diabetes Research Foundation. (2015). *Pilot clinical trial of Ustekinumab in patients with new-onset T1D (UST1D)*. https://clinicaltrials.gov/ct2/show/NCT02117765

Juvenile Diabetes Research Foundation, & Janssen, L. P. (2019). *Clinical phase II/III trial of ustekinumab to treat type 1 diabetes (UST1D2) (UST1D2)*. https://clinicaltrials.gov/ct2/show/NCT03941132

Kalafat, E., Sukur, Y. E., Abdi, A., Thilaganathan, B., & Khalil, A. (2018). Metformin for prevention of hypertensive disorders of pregnancy in women with gestational diabetes or

obesity: Systematic review and meta-analysis of randomized trials. *Ultrasound in Obstetrics & Gynecology*, *52*(6), 706-714.

Karamanou, M., Protogerou, A., Tsoucalas, G., Androutsos, G., & Poulakou-Rebelakou, E. (2016). Milestones in the history of diabetes mellitus: The main contributors. *World Journal of Diabetes*, *7*(1), 1.

Kassir, Z., Sarpatwari, A., Kocak, B., Kuza, C. C., & Gellad, W. F. (2020). Sponsorship and funding for gene therapy trials in the United States. *JAMA*, *323*(9), 890-891.

Kellogg Eye Center. (n.d.). *Retinal neovascularization*. http://kellogg.umich.edu/theeyeshaveit/opticfundus/retinal_neovascularization.html

Kessler, J. A., Shaibani, A., Sang, C. N., Christiansen, M., Kudrow, D., Vinik, A., & Shin, N. (2021). Gene therapy for diabetic peripheral neuropathy: A randomized, placebo-controlled phase III study of VM202, a plasmid DNA encoding human hepatocyte growth factor. *Clinical and Translational Science*, *14*(3), 1176-1184.

Kessler, J. A., Shaibani, A., Sang, C. N., Christiansen, M., Kudrow, D., Vinik, A., Shin, N., Ajroud-Driss, S., Allen, J., Biton, V., Bowman, M., Brannagan, T., Chehade, J., Cherlin, R. S., Dimachkie, M., Gimbel, J. S., Kasper, M., Klaff, L., Lynn, L. D., . . . Ziedalski, T. (2021). Gene therapy for diabetic peripheral neuropathy: A randomized, placebo-controlled phase III study of VM202, a plasmid DNA encoding human hepatocyte growth factor [Article]. *Clinical and Translational Science*, *14*(3), 1176-1184. https://doi.org/10.1111/cts.12977

Kessler, J. A., Shaibani, A., Sang, C. N., Christiansen, M., Kudrow, D., Vinik, A., Shin, N., & group, t. V. s. (2021). Gene therapy for diabetic peripheral neuropathy: A randomized, placebo-controlled phase III study of VM202, a plasmid DNA encoding human

hepatocyte growth factor. *Clinical and Translational Science*, *14*(3), 1176-1184. https://doi.org/https://doi.org/10.1111/cts.12977

Kessler, J. A., Smith, A. G., Cha, B.-S., Choi, S. H., Wymer, J., Shaibani, A., Ajroud-Driss, S., Vinik, A., & Group, V. D.-I. S. (2015). Double-blind, placebo-controlled study of HGF gene therapy in diabetic neuropathy. *Annals of Clinical and Translational Neurology*, *2*(5), 465-478. https://doi.org/10.1002/acn3.186

Kessler, J. A., Smith, A. G., Cha, B. S., Choi, S. H., Wymer, J., Shaibani, A., Ajroud-Driss, S., & Vinik, A. (2015). Double-blind, placebo-controlled study of HGF gene therapy in diabetic neuropathy [Article]. *Annals of Clinical and Translational Neurology*, *2*(5), 465-478. https://doi.org/10.1002/acn3.186

Kim, J. W., Luo, J. Z., & Luo, L. (2019). Bone marrow mesenchymal stem cells as a new therapeutic approach for diabetes mellitus. In *A roadmap to non-hematopoietic stem cell-based therapeutics* (pp. 251-273). Elsevier.

Kiyosue, A., Hayashi, N., Komori, H., Leonsson-Zachrisson, M., & Johnsson, E. (2013). Dose-ranging study with the glucokinase activator AZD1656 as monotherapy in Japanese patients with type 2 diabetes mellitus. *Diabetes, Obesity and Metabolism*, *15*(10), 923-930.

Kleinberger, J. W., & Pollin, T. I. (2015). Personalized medicine in diabetes mellitus: Current opportunities and future prospects. *Annals of the New York Academy of Sciences*, *1346*(1), 45.

Kusumanto, Y. H., Van Weel, V., Mulder, N. H., Smit, A. J., Van Den Dungen, J. J. A. M., Hooymans, J. M. M., Sluiter, W. J., Tio, R. A., Quax, P. H. A., Gans, R. O. B., Dullaart, R. P. F., & Hospers, G. A. P. (2006). Treatment with intramuscular vascular endothelial

growth factor gene compared with placebo for patients with diabetes mellitus and critical limb ischemia: A double-blind randomized trial. *Human Gene Therapy*, *17*(6), 683-691. https://doi.org/10.1089/hum.2006.17.683

Laird, E. A., & Coates, V. (2013). Systematic review of randomized controlled trials to regulate glycaemia after stroke. *Journal of Advanced Nursing 69*(2), 263-277.

Langenberg, C., & Lotta, L. A. (2018). Genomic insights into the causes of type 2 diabetes. *The Lancet*, *391*(10138), 2463-2474.

Lazzarini, P., Pacella, R. E., Armstrong, D., & Van Netten, J. (2018). Diabetes-related lower-extremity complications are a leading cause of the global burden of disability. *Diabetic Medicine*, *35*(9), 1297-1299.

Lin, X., Xu, Y., Pan, X., Xu, J., Ding, Y., Sun, X., Song, X., Ren, Y., & Shan, P.-F. (2020). Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. *Scientific Reports*, *10*(1), 1-11.

Linares-Espinós, E., Hernández, V., Domínguez-Escrig, J., Fernández-Pello, S., Hevia, V., Mayor, J., Padilla-Fernández, B., & Ribal, M. (2018). Methodology of a systematic review. *Actas Urológicas Españolas (English Edition)*, *42*(8), 499-506.

Ling, S., Brown, K., Miksza, J. K., Howells, L., Morrison, A., Issa, E., Yates, T., Khunti, K., Davies, M. J., & Zaccardi, F. (2020). Association of type 2 diabetes with cancer: A meta-analysis with bias analysis for unmeasured confounding in 151 cohorts comprising 32 million people. *Diabetes Care*, *43*(9), 2313-2322.

Livinski, A. (2015). Undertaking a systematic review: What you need to know. *National Institutes of Health Library.*

Lu, R.-M., Hwang, Y.-C., Liu, I.-J., Lee, C.-C., Tsai, H.-Z., Li, H.-J., & Wu, H.-C. (2020). Development of therapeutic antibodies for the treatment of diseases. *Journal of Biomedical Science, 27*(1), 1-30.

Lyerla, R., Johnson-Rabbett, B., Shakally, A., Magar, R., Alameddine, H., & Fish, L. (2021). Recurrent DKA results in high societal costs–a retrospective study identifying social predictors of recurrence for potential future intervention. *Clinical Diabetes and Endocrinology, 7*(1), 1-6.

Ma, C.-C., Wang, Z.-L., Xu, T., He, Z.-Y., & Wei, Y.-Q. (2020). The approved gene therapy drugs worldwide: from 1998 to 2019. *Biotechnology Advances, 40*, 107502.

Ma, S., Viola, R., Sui, L., Cherubini, V., Barbetti, F., & Egli, D. (2018). β cell replacement after gene editing of a neonatal diabetes-causing mutation at the insulin locus. *Stem Cell Reports, 11*(6), 1407-1415.

Marcovecchio, M. L., Wicker, L. S., Dunger, D. B., Dutton, S. J., Kopijasz, S., Scudder, C., Todd, J. A., & Johnson, P. R. (2020). Interleukin-2 therapy of autoimmunity in diabetes (ITAD): A phase 2, multicentre, double-blind, randomized, placebo-controlled trial. *Wellcome Open Research, 5*.

Marwaha, A. K., Chow, S., Pesenacker, A. M., Cook, L., Sun, A., Long, S. A., Yang, J. H., Ward-Hartstonge, K. A., Williams, E., & Domingo-Vila, C. (2022). A phase 1b open-label dose-finding study of ustekinumab in young adults with type 1 diabetes. *Immunotherapy Advances, 2*(1), ltab022.

Masic, I., & Milinovic, K. (2012). On-line biomedical databases-the best source for quick search of the scientific information in the biomedicine. *Acta Informatica Medica: AIM: Journal*

of the Society for Medical Informatics of Bosnia & Herzegovina: Casopis Drustva za Medicinsku Informatiku BiH, *20*(2), 72-84.

Matharoo, K., Singh, Y., Sokhi, J., Sharma, R., Raina, P., Kaur, R., Sikka, R., Kaul, N., & Bhanwer, A. (2017). Molecular genetics of type 2 diabetes: A review. *Human Genomics and Applications*, 179-221.

Matsumoto, S., Abalovich, A., Wechsler, C., Wynyard, S., & Elliott, R. B. (2016). Clinical benefit of islet xenotransplantation for the treatment of type 1 diabetes. *EBioMedicine*, *12*, 255-262.

Matsumoto, S., Tan, P., Baker, J., Durbin, K., Tomiya, M., Azuma, K., Doi, M., & Elliott, R. (2014). Clinical porcine islet xenotransplantation under comprehensive regulation. *Transplantation Proceedings*, *46*(6), 1992-1995.

Matsumoto, S., Tan, P., Baker, J., Durbin, K., Tomiya, M., Azuma, K., & Elliott, R. (2014). Clinical porcine islet xenotransplantation under comprehensive regulation. *Transplantation Proceedings*, *46*(6), 1992-1995.

Maulik, N. (2009). NV1FGF, a pCOR plasmid-based angiogenic gene therapy for the treatment of intermittent claudication and critical limb ischemia. *Current Opinion in Investigational Drugs (London, England: 2000)*, *10*(3), 259-268.

Maxwell, K. G., Augsornworawat, P., Velazco-Cruz, L., Kim, M. H., Asada, R., Hogrebe, N. J., Morikawa, S., Urano, F., & Millman, J. R. (2020). Gene-edited human stem cell–derived β cells from a patient with monogenic diabetes reverse preexisting diabetes in mice, *Science Translational Medicine, 12(*540).

McHugh, M. L. (2012). Interrater reliability: The kappa statistic. *Biochemia Medica*, *22*(3), 276-282.

Meert, D., Torabi, N., & Costella, J. (2016). Impact of librarians on reporting of the literature searching component of pediatric systematic reviews. *Journal of the Medical Library Association*, *104*(4), 267.

Merck & Co. (2022). Overview of B- and T-Cell function. https://www.msdmanuals.com/professional/multimedia/video/overview-of-b-and-t-cell-function

Metzendorf, M.-I. (2016). Why medical information specialists should routinely form part of teams producing high quality systematic reviews–a Cochrane perspective. *Journal of the European Association for Health Information and Libraries*, *12*(4), 6-9.

Mousa, A., Naderpoor, N., Teede, H., Scragg, R., & Courten, B. D. (2018). Vitamin D supplementation for improvement of chronic low-grade inflammation in patients with type 2 diabetes: A systematic review and meta-analysis of randomized controlled trials. *Nutrition Reviews*, *76*(5), 380-394.

National Academies of Sciences, Engineering, & Medicine. (2017). *Human genome editing: Science, ethics, and governance*. National Academies Press.

National Cancer Institute. (2021). *CAR T-cell therapy*. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/car-t-cell-therapy

National Center for Advancing Translational Sciences. (2021). *Maternally inherited diabetes and deafness*. https://rarediseases.info.nih.gov/diseases/4003/maternally-inherited-diabetes-and-deafness

National Center for Biotechnology Information. (2021). *Thiazolidinediones*. https://www.ncbi.nlm.nih.gov/books/NBK551656/

National Center for Biotechnology Information. (2022). *Physiology: Growth factor*. https://www.ncbi.nlm.nih.gov/books/NBK442024/

National Center for Biotechnology Information. (n.d.). *Bookshelf appendix F : Quality rating form*. https://www.ncbi.nlm.nih.gov/books/NBK56923/#jadad

National Eye Institute. (2022a). *Age-related macular degeneration (AMD)*. https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/age-related-macular-degeneration

National Eye Institute. (2022b). Diabetic retinopathy. https://www.nei.nih.gov/learn-about-eye-health/eye-conditions-and-diseases/diabetic-retinopathy

National Human Genome Research Institute. (2021). *Genetic engineering*. https://www.genome.gov/genetics-glossary/Genetic-Engineering

National Human Genome Research Institute. (n.d.). *Autosomal dominant*. https://www.genome.gov/genetics-glossary/Autosomal-Dominant

National Insititutes of Health. (2021). *Alpha cells conversion to beta cells in non-human primates*. https://grantome.com/grant/NIH/R01-DK120377-01

National Institute of Diabetes and Digestive and Kidney Diseases. (2016). *Insulin, medicines, & other diabetes treatments*. https://www.niddk.nih.gov/health-information/diabetes/overview/insulin-medicines-treatments#:~:text=Most%20people%20with%20type%202,a%20small%20amount%20of%20weight.

National Institute of Diabetes and Digestive and Kidney Diseases. (2019). *Cellular therapy for type 1 diabetes using mesenchymal stem cells*. https://clinicaltrials.gov/ct2/show/NCT04061746

National Institutes of Health. (2021). *Pancreatic islet transplantation*. https://www.niddk.nih.gov/health-information/diabetes/overview/insulin-medicines-treatments/pancreatic-islet-transplantation

National Institutes of Health. (2022). *Anti-SARS-CoV-2 monoclonal antibodies*. https://www.covid19treatmentguidelines.nih.gov/therapies/anti-sars-cov-2-antibody-products/anti-sars-cov-2-monoclonal-antibodies/

National Institutes of Health Library. (2022). *Systematic reviews: Tools and resources.* https://www.nihlibrary.nih.gov/services/systematic-review-service/tools-resources

National Library of Medicine. (2020). *What are genome editing and CRISPR-Cas9?* https://medlineplus.gov/genetics/understanding/genomicresearch/genomeediting/

Nature Medicine. (2021). Gene therapy needs a long-term approach. *Nature Medicine*, *27*(4), 563-563. https://doi.org/10.1038/s41591-021-01333-6

Nguyen, L. T., Hoang, D. M., Nguyen, K. T., Bui, D. M., Nguyen, H. T., Le, H. T., Hoang, V. T., Bui, H. T., Dam, P. T., & Hoang, X. T. (2021). Type 2 diabetes mellitus duration and obesity alter the efficacy of autologously transplanted bone marrow-derived mesenchymal stem/stromal cells. *Stem Cells Translational Medicine*. *10*(9), 1266-1278.

Nóbrega, C., Mendonça, L., & Matos, C. A. (2020). Gene therapy strategies. In *A handbook of gene and cell therapy*. Springer.

Northeastern University. (n.d.). *Central dogma of molecular biology*. https://web.northeastern.edu/bbarbiellini/CBIO3580/DOGMA/DNA_CenDog.html#6.%20Mutations

Osipovich, A. B., & Magnuson, M. A. (2018). Alpha to beta cell reprogramming: Stepping toward a new treatment for diabetes. *Cell Stem Cell*, *22*(1), 12-13.

Paez, A. (2017). Gray literature: An important resource in systematic reviews. *Journal of Evidence-Based Medicine*, *10*(3), 233-240.

Patil, S. J., Ruppar, T., Koopman, R. J., Lindbloom, E. J., Elliott, S. G., Mehr, D. R., & Conn, V. S. (2018). Effect of peer support interventions on cardiovascular disease risk factors in adults with diabetes: A systematic review and meta-analysis. *BMC Public Health*, *18*(1), 398.

Piasecki, J., Waligora, M., & Dranseika, V. (2018). Google search as an additional source in systematic reviews. *Science and Engineering Ethics*, *24*(2), 809-810.

Piñero, J., Furlong, L. I., & Sanz, F. (2018). In silico models in drug development: Where we are. *Current Opinion in Pharmacology*, *42*, 111-121.

Pollock, A., & Berge, E. (2018). How to do a systematic review. *International Journal of Stroke*, *13*(2), 138-156.

Porrett, P. M., Orandi, B. J., Kumar, V., Houp, J., Anderson, D., Cozette Killian, A., Hauptfeld-Dolejsek, V., Martin, D. E., Macedon, S., & Budd, N. (2022). First clinical-grade porcine kidney xenotransplant using a human decedent model. *American Journal of Transplantation*, *22*(4), 1037-1053.

Prasad, R. B., & Groop, L. (2015). Genetics of type 2 diabetes—Pitfalls and possibilities. *Genes*, *6*(1), 87-123.

Precigen Actobio. (2018). *A study to assess the safety and tolerability of different doses of AG019 administered alone or in combination with teplizumab in participants with recently diagnosed type 1 diabetes mellitus (T1D).* https://clinicaltrials.gov/ct2/show/NCT03751007

Precigen Actobio. (2021). *Precigen inverstors press releases*. https://investors.precigen.com/news-releases/news-release-details/precigen-actobio-announces-additional-positive-interim-data

Prokosch, V., Stupp, T., Spaniol, K., Pham, E., & Nikol, S. (2014). Angiogenic gene therapy does not cause retinal pathology. *The Journal of Gene Medicine*, *16*(9-10), 309-316.

ProventionBio. (2022). *Provention Bio announces extension of FDA user fee goal date for Teplizumab to November 17, 2022*. https://investors.proventionbio.com/2022-06-30-Provention-Bio-Announces-Extension-of-FDA-User-Fee-Goal-Date-for-Teplizumab-to-November-17,-2022

Qadir, M. M. F., Lanzoni, G., Ricordi, C., & Domínguez-Bendala, J. (2020). Human pancreatic progenitors. In *Transplantation, bioengineering, and regeneration of the endocrine pancreas* (pp. 183-200). Elsevier.

Qazi, Y., Maddula, S., & Ambati, B. K. (2009). Mediators of ocular angiogenesis. *Journal of Genetics*, *88*(4), 495-515.

Rabin, R. (2022). Kidneys from a genetically altered pig are implanted in a brain-dead patient. *The New York Times*, *20*.

Rajkumar, V., & Levine, S. N. (2020). Latent autoimmune diabetes. In *StatPearls*. StatPearls Publishing.

Rakoczy, E., Magno, A., Lai, C.-M., Degli-Esposti, M., & Constable, I. (2018). Subanalysis of data from rAAV.sFLT-1 phase 1 and 2a randomized gene therapy trials for wet age-related macular degeneration. *Investigative Ophthalmology & Visual Science*, *59*(9), 1448-1448.

Rakoczy, E. P., Lai, C.-M., Magno, A., French, M., Butler, S., Barone, S., Schwartz, S. D., Blumenkranz, M., Degli-Esposti, M., & Constable, I. (2017). Safety and post hoc analysis of subretinal rAAV.sFLT-1 for wet age-related macular degeneration following a phase 2a randomized clinical trial. *Investigative Ophthalmology & Visual Science*, *58*(8), 4476-4476.

Rakoczy, E. P., Lai, C. M., Magno, A. L., Wikstrom, M. E., French, M. A., Pierce, C. M., Schwartz, S. D., Blumenkranz, M. S., Chalberg, T. W., Degli-Esposti, M. A., & Constable, I. J. (2015). Gene therapy with recombinant adeno-associated vectors for neovascular age-related macular degeneration: 1 year follow-up of a phase 1 randomised clinical trial. *Lancet*, *386*(10011), 2395-2403. https://doi.org/10.1016/s0140-6736(15)00345-1

Rakoczy, E. P., Magno, A. L., Lai, C.-M., Pierce, C. M., Degli-Esposti, M. A., Blumenkranz, M. S., & Constable, I. J. (2019). Three-year follow-up of phase 1 and 2a rAAV. sFLT-1 subretinal gene therapy trials for exudative age-related macular degeneration. *American Journal of Ophthalmology*, *204*, 113-123.

Redondo, M. J., Steck, A. K., & Pugliese, A. (2018). Genetics of type 1 diabetes. *Pediatric Diabetes*, *19*(3), 346-353.

REGENXBIO, Inc. (2020). *RGX-314 gene therapy administered in the suprachoroidal space for participants with diabetic retinopathy (DR) without center involved-diabetic macular edema (CI-DME) (ALTITUDE)*. https://clinicaltrials.gov/ct2/show/NCT04567550

REGENXBIO, Inc. (2022). *REGENXBIO presents positive interim data from phase II altitude™ trial of rgx-314 for the treatment of diabetic retinopathy using suprachoroidal delivery*.

https://regenxbio.gcs-web.com/news-releases/news-release-details/regenxbio-presents-positive-interim-data-phase-ii-altitudetm

Rethlefsen, M. L., Farrell, A. M., Trzasko, L. C. O., & Brigham, T. J. (2015). Librarian co-authors correlated with higher quality reported search strategies in general internal medicine systematic reviews. *Journal of Clinical Epidemiology*, *68*(6), 617-626.

Robinson, K. N., Terrazas, S., Giordano-Mooga, S., & Xavier, N. A. (2020). The role of heteroplasmy in the diagnosis and management of maternally inherited diabetes and deafness. *Endocrine Practice*, *26*(2), 241-246.

Roep, B. O., Solvason, N., Gottlieb, P. A., Abreu, J. R., Harrison, L. C., Eisenbarth, G. S., Yu, L., Leviten, M., Hagopian, W. A., & Buse, J. B. (2013). Plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific CD8+ T cells in type 1 diabetes. *Science Translational Medicine*, *5*(191), 191ra182-191ra182.

Ropper, A. H., Gorson, K. C., Gooch, C. L., Weinberg, D. H., Pieczek, A., Ware, J. H., Kershen, J., Rogers, A., Simovic, D., & Schratzberger, P. (2009). Vascular endothelial growth factor gene transfer for diabetic polyneuropathy: a randomized, double-blinded trial. *Annals of Neurology*, *65*(4), 386-393.

Rosenzwajg, M., Salet, R., Lorenzon, R., Tchitchek, N., Roux, A., Bernard, C., Carel, J.-C., Storey, C., Polak, M., & Beltrand, J. (2020). Low-dose IL-2 in children with recently diagnosed type 1 diabetes: a Phase I/II randomised, double-blind, placebo-controlled, dose-finding study. *Diabetologia*, *63*(9), 1808-1821.

Rosik, J., Szostak, B., Machaj, F., & Pawlik, A. (2020). The role of genetics and epigenetics in the pathogenesis of gestational diabetes mellitus. *Annals of Human Genetics*, *84*(2), 114-124.

Rothblatt, M. (2022). Commentary on achievement of first life-saving xenoheart transplant. *Xenotransplantation*, e12746.

Seelig et al. (2018). The DILfrequency study is an adaptive trial to identify optimal IL-2 dosing in patients with type 1 diabetes. *JCI Insight*, *3*(19).

Shapiro, A. J. (2018). Gearing up for stem cell-derived beta cells—Are we ready? *Transplantation*, *102*(8), 1207-1208.

Shenzhen SiBiono GeneTech Co., Ltd. (2015). *p53 gene therapy in treatment of diabetes concurrent with hepatocellular carcinoma*. https://clinicaltrials.gov/show/NCT02561546

Shishehbor, M. H., Rundback, J., Bunte, M., Hammad, T. A., Miller, L., Patel, P. D., Sadanandan, S., Fitzgerald, M., Pastore, J., Kashyap, V., & Henry, T. D. (2019). SDF-1 plasmid treatment for patients with peripheral artery disease (STOP-PAD): Randomized, double-blind, placebo-controlled clinical trial. *Vascular Medicine*, *24*(3), 200-207. https://doi.org/10.1177/1358863x18817610

Shoily, S. S., Ahsan, T., Fatema, K., & Sajib, A. A. (2021). Common genetic variants and pathways in diabetes and associated complications and vulnerability of populations with different ethnic origins. *Scientific Reports*, *11*(1), 1-14.

Silva, G. B. d., Oliveira, J. G. R. d., Oliveira, M. R. B. d., Vieira, L. J. E. d. S., & Dias, E. R. (2018). Global costs attributed to chronic kidney disease: a systematic review. *Revista da Associação Médica Brasileira*, *64*, 1108-1116.

Simon, F., Duran, M., Garabet, W., Schelzig, H., Jacobs, M., & Gombert, A. (2022). Gene therapy of chronic limb-threatening ischemia: Vascular medical perspectives. *Journal of Clinical Medicine*, *11*(5), 1282.

Stewart, A. F. (2021). Human beta cell regenerative drug therapy for diabetes: Past achievements and future challenges. *Frontiers in Endocrinology*, *12*, 776.

Suksomboon, N., Poolsup, N., & Sinprasert, S. (2011). Effects of vitamin E supplementation on glycaemic control in type 2 diabetes: Systematic review of randomized controlled trials. *Journal of Clinical Pharmacy & Therapeutics*, *36*(1), 53-63.

Tamura, R., & Toda, M. (2020). Historic overview of genetic engineering technologies for human gene therapy. *Neurologia Medico-Chirurgica*, ra. 2020-0049.

Tattersall, R. B. (2017). The history of diabetes mellitus. *Textbook of Diabetes*, 1-22.

Teng, D., Gong, Y., Wu, Z., Li, W., Tang, Y., & Liu, G. (2021). In silico prediction of potential drug combinations for type 2 diabetes mellitus by an integrated network and transcriptome analysis. *ChemMedChem*, *16*, 1-11.

Terens, N., Vecchi, S., Bargagli, A. M., Agabiti, N., Mitrova, Z., Amato, L., & Davoli, M. (2018). Quality improvement strategies at primary care level to reduce inequalities in diabetes care: An equity-oriented systematic review. *BMC Endocrine Disorders*, *18*(1).

The American Journal of Managed Care. (2014). *New orphan drug in the t1dm realm: Another immunotherapy success story?* https://www.ajmc.com/view/new-orphan-drug-in-the-t1dm-realm-another-immunotherapy-success-story

The National Center for Biotechnology Information. (2017). *Selection of tools for appraising evidence*. https://www.ncbi.nlm.nih.gov/books/NBK453537/

The National Institute of Diabetes and Digestive and Kidney Diseases. (2022). *Diabetic neuropathy*. https://www.niddk.nih.gov/health-information/diabetes/overview/preventing-problems/nerve-damage-diabetic-neuropathies

Thomas, E. R., Brackenridge, A., Kidd, J., Kariyawasam, D., Carroll, P., Colclough, K., & Ellard, S. (2016). Diagnosis of monogenic diabetes: 10-Year experience in a large multi-ethnic diabetes center. *Journal of Diabetes Investigation*, *7*(3), 332-337.

Todd, J. A., Evangelou, M., Cutler, A. J., Pekalski, M. L., Walker, N. M., Stevens, H. E., Porter, L., Smyth, D. J., Rainbow, D. B., & Ferreira, R. C. (2016a). Regulatory T cell responses in participants with type 1 diabetes after a single dose of interleukin-2: A non-randomised, open label, adaptive dose-finding trial. *PLoS medicine*, *13*(10), e1002139.

Todd, J. A., Evangelou, M., Cutler, A. J., Pekalski, M. L., Walker, N. M., Stevens, H. E., Porter, L., Smyth, D. J., Rainbow, D. B., & Ferreira, R. C. (2016b). Regulatory T cell responses in participants with type 1 diabetes after a single dose of interleukin-2: A non-randomised, open label, adaptive dose-finding trial. *PLoS Medicine*, *13*(10), e1002139.

Tolerion, I. (2019). *Diabetes autoimmunity withdrawn in new onset and in established patients (SUNRISE)*. https://clinicaltrials.gov/show/NCT03895437

Tufts University Library. (2022). *Grey literature for systematic reviews*. https://researchguides.library.tufts.edu/c.php?g=249130&p=1658806

UCSF. (2022). *Diabetes education online*. https://dtc.ucsf.edu/types-of-diabetes/type1/understanding-type-1-diabetes/autoimmunity/what-causes-autoimmune-diabetes/

Ukraine Association of Biobank. (2020). *Longterm follow-up of subjects with diabetes 2 type treatment with ex vivo gene therapy (AUB001)*. https://clinicaltrials.gov/show/NCT04642911

University of Alberta. (2022a). *cePolyTregs in islet transplantation (cePolyTregs)*. https://clinicaltrials.gov/show/NCT05349591

University of Alberta. (2022b). *History of a diabetes breakthrough*. https://www.ualberta.ca/medicine/momentum/2020/momentum-stories-2020/2020/may/history-of-a-diabetes-breakthrough.html

University of Colorado, Denver. (2022). *MER3101: MAS-1 adjuvanted antigen-specific immunotherapeutic for prevention and treatment of type 1 diabetes (MER3101)*. University of Colorado, Denver.

University of Rochester. (2021). *Diabetes during pregnancy*. https://www.urmc.rochester.edu/encyclopedia/content.aspx?ContentTypeID=90&ContentID=P02444

Urakami, T. (2019). Maturity-onset diabetes of the young (MODY): Current perspectives on diagnosis and treatment. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 12, 1047.

U.S. Food and Drug Administration. (2018). *What is gene therapy?* https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/what-gene-therapy

U.S. Food and Drug Administration. (2019a). *FDA approves first oral GLP-1 treatment for type 2 diabetes.* https://www.fda.gov/news-events/press-announcements/fda-approves-first-oral-glp-1-treatment-type-2-diabetes

U.S. Food and Drug Administration. (2019b). *FDA approves new treatment for pediatric patients with type 2 diabetes.* https://www.fda.gov/news-events/press-announcements/fda-approves-new-treatment-pediatric-patients-type-2-diabetes

U.S. Food and Drug Administration. (2021a). *Cellular & gene therapy products*. https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products

U.S. Food and Drug Administration. (2021b). *Orphan drug designations and approvals*. https://www.accessdata.fda.gov/scripts/opdlisting/oopd/detailedIndex.cfm?cfgridkey=100696

Vaillant, A. A. J., & Qurie, A. (2021). Interleukin. In *StatPearls [Internet]*. StatPearls Publishing.

Vinmec Research Institute of Stem Cell and Gene Technology. (2017). *Outcomes of expanded autologous bone marrow-derived mesenchymal stem cells therapy in type II diabetes (ASD2)*. https://clinicaltrials.gov/show/NCT03343782

Voglová, B., Zahradnická, M., Girman, P., Kríž, J., Berková, Z., Koblas, T., Vávrová, E., Németová, L., Kosinová, L., & Habart, D. (2017). Benefits of islet transplantation as an alternative to pancreas transplantation: Retrospective study of more than 10 Ten years of experience in a single center. *The Review of Diabetic Studies: RDS*, *14*(1), 10.

White, J. R. (2014). A brief history of the development of diabetes medications. *Diabetes Spectrum*, *27*(2), 82-86.

Williams, R., Karuranga, S., Malanda, B., Saeedi, P., Basit, A., Besançon, S., Bommer, C., Esteghamati, A., Ogurtsova, K., & Zhang, P. (2020). Global and regional estimates and projections of diabetes-related health expenditure: Results from the International Diabetes Federation Diabetes Atlas. *Diabetes Research and Clinical Practice*, 162, 108072.

World Health Organization. (2018a). *Genetics and diabetes*. https://www.who.int/genomics/about/Diabetis-fin.pdf

World Health Organization. (2018b). *Global health expenditure database*. https://apps.who.int/nha/database

Yahaya, T., & Salisu, T. (2021). Genes predisposing to type 1 diabetes mellitus and pathophysiology: A narrative review. *arXiv preprint arXiv:2101.06680*.

Ylä-Herttuala, S. (2019). Gene therapy of critical limb ischemia enters clinical use. *Molecular Therapy*, *27*(12), 2053.

Yu, X., Chau, J. P. C., & Huo, L. (2018). The effectiveness of traditional Chinese medicine-based lifestyle interventions on biomedical, psychosocial, and behavioral outcomes in individuals with type 2 diabetes: A systematic review with meta-analysis. *International Journal of Nursing Studies*, *80*, 165-180.

Zhang, J., Liu, H., Luo, S., Lu, Z., Chávez-Badiola, A., Liu, Z., Yang, M., Merhi, Z., Silber, S. J., & Munné, S. (2017). Live birth derived from oocyte spindle transfer to prevent mitochondrial disease. *Reproductive Biomedicine Online*, *34*(4), 361-368.

Zhang, P., Lu, J., Jing, Y., Tang, S., Zhu, D., & Bi, Y. (2017). Global epidemiology of diabetic foot ulceration: A systematic review and meta-analysis. *Annals of Medicine*, *49*(2), 106-116.

Zhang, W.-W., Li, L., Li, D., Liu, J., Li, X., Li, W., Xu, X., Zhang, M. J., Chandler, L. A., & Lin, H. (2018). The first approved gene therapy product for cancer Ad-p53 (Gendicine): 12 years in the clinic. *Human Gene Therapy*, *29*(2), 160-179.

Zheng, Y., Ley, S. H., & Hu, F. B. (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology*, *14*(2), 88-98.

Zhu, Y., Sidell, M. A., Arterburn, D., Daley, M. F., Desai, J., Fitzpatrick, S. L., Horberg, M. A., Koebnick, C., McCormick, E., & Oshiro, C. (2019). Racial/ethnic disparities in the prevalence of diabetes and prediabetes by BMI: Patient Outcomes Research To Advance

Learning (PORTAL) multisite cohort of adults in the US. *Diabetes Care*, *42*(12), 2211-2219.

Appendices

Appendix A: Database Searches

1) **PubMed Search:**

   Search string: ("diabetes"[Title/Abstract] AND "gene therapy"[Title/Abstract]) AND ((clinicalstudy[Filter] OR clinicaltrial[Filter] OR clinicaltrialphasei[Filter] OR clinicaltrialphaseii[Filter] OR clinicaltrialphaseiii[Filter] OR clinicaltrialphaseiv[Filter] OR controlledclinicaltrial[Filter] OR randomizedcontrolledtrial[Filter]) AND (humans[Filter] OR animal[Filter]) AND (english[Filter]) AND (2000:2022[pdat]))

2) **Embase Search:**

   Search string: ('diabetes mellitus'/exp OR 'diabetes' OR 'diabetes mellitus' OR 'diabetic') AND ('gene therapy'/exp OR 'gene therapies' OR 'gene therapy' OR 'gene treatment' OR 'genetic therapy') AND ('randomized controlled trial'/exp OR 'controlled trial, randomized' OR 'randomised controlled study' OR 'randomised controlled trial' OR 'randomized controlled study' OR 'randomized controlled trial') AND (2000:py OR 2001:py OR 2003:py OR 2005:py OR 2006:py OR 2007:py OR 2008:py OR 2009:py OR 2010:py OR 2011:py OR 2012:py OR 2013:py OR 2014:py OR 2015:py OR 2016:py OR 2017:py OR 2018:py OR 2019:py OR 2020:py OR 2021:py OR 2022:py) AND 'article'/it

3) **Web of Science Search:**

   Search string: **diabetes* AND gene therapy* AND controlled* AND randomized* NOT mice* NOT rat* NOT rodent* NOT cancer*** (All Fields) and **Articles** (Document Types) and **Articles** (Document Types) and **Gastroenterology Hepatology** or **Cardiac Cardiovascular Systems** or **Pharmacology Pharmacy** or **Infectious Diseases** or **Critical Care**

Medicine or **Medical Laboratory Technology** or **Nutrition Dietetics** or **Public Environmental Occupational Health** or **Respiratory System** or **Surgery** (Exclude – Web of Science Categories) and **Hematology** (Exclude – Web of Science Categories).

4) **Scopus Search:**

Search string:

(TITLE (diabetes* AND gene AND therapy*) AND PUBYEAR > 2000 AND PUBYEAR < 2022) AND (control) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (SRCTYPE , "j")) AND (EXCLUDE (SUBJAREA , "PHAR") OR EXCLUDE (SUBJAREA , "AGRI") OR EXCLUDE (SUBJAREA , "NURS") OR EXCLUDE (SUBJAREA , "CHEM") OR EXCLUDE (SUBJAREA , "VETE")) AND (EXCLUDE (EXACTSRCTITLE , "Journal Of Sexual Medicine") OR EXCLUDE (EXACTSRCTITLE , "Lijecnicki Vjesnik") OR EXCLUDE (EXACTSRCTITLE , "Acta Medica Mediterranea") OR EXCLUDE (EXACTSRCTITLE , "Acta Physiologica Sinica") OR EXCLUDE (EXACTSRCTITLE , "Medecine Sciences") OR EXCLUDE (EXACTSRCTITLE , "Medizinische Welt") OR EXCLUDE (EXACTSRCTITLE , "Problemy Endokrinologii") OR EXCLUDE (EXACTSRCTITLE , "Terapevticheskii Arkhiv") OR EXCLUDE (EXACTSRCTITLE , "Voprosy Pitaniia") OR EXCLUDE (EXACTSRCTITLE , "World Journal Of Gastroenterology")) AND (LIMIT-TO (EXACTKEYWORD , "Article") OR LIMIT-

TO (EXACTKEYWORD , "Controlled Study")) AND (LIMIT-

TO (LANGUAGE , "English")) AND (LIMIT-TO (EXACTKEYWORD , "Gene

Therapy"))

5) **CENTRAL Search**
Search string: “diabetes” AND “gene therapy”
Search limit
For Content: Trials
Search limit
For publication year: Between 2000 and 2022

6) **Clinicaltrials.gov Search**

Condition: diabetes
Other terms (NCT number, drug name, investigator name): gene therapy

7) **WHO Clinical Trials Site Search:**

Search string: “diabetes” AND “gene therapy”

8) **International Manufacturers Site Search:**

Products: “gene therapy”

9) **GreyNet Search:**

Search string: “diabetes” AND gene “therapy”

10) **US Federal Science Alliance Search:**

Search string: “diabetes” AND “gene therapy” Date Range 2000 to 2021. Categories:

Science.gov Websites - Selected Websites, Applied Science & Technologies -

Biotechnology, Electronics, Engineering, Transport, Biology & Nature - Animals &

Plants, Ecology, Genetics, Pest Control, General Science - Multidisciplinary resources,

Health & Medicine - Disease, Health Care, Nutrition, Mental Health, Public Access -

Peer-reviewed scholarly publications resulting from federally funded scientific research.

Appendix B: Coding Protocol and Kappa Calculation

Coding Protocol for Interrater Reliability Created Based on Predetermined Inclusion Criteria Outlined in the Proposal

Studies were assigned a "Yes" based on the following inclusion criteria

1) Date: January 2000 to December 2021
2) Language: English
3) Study design: Interventional or follow-up of an intervention
4) Study topic: Gene modification/gene expression/gene addition/gene deletion/ gene silencing/genetically engineered expanded or enhanced cell therapy for any type of diabetes and/or diabetes-related complications

Studies were assigned a "No" based on the following exclusion criteria

1) Dates: Before January 2000 or after December 2021
2) Language: Any language other than English
3) Study design: Any study design other than interventional or follow-up of an intervention
4) Study topic: any other therapy that does not involve gene modification/gene expression/gene addition/gene deletion/ gene silencing/genetically engineered expanded or enhanced cell therapy for any type of diabetes and/or diabetes-related complications such as nutritional supplements or bariatric surgery

The number of 'Yes" and "No" were tallied and filled into a 2x2 table.

Researcher1				
Reviewer		Yes	No	Total
	Yes	47	50	97
	No	4	1	5
	Total	51	51	

Observed agreement: (both said "yes" + both said "no")/total number of ratings= (47+1)/51=0.94
Expected agreement: (97/100*51/100) +(5/100*51/100) =0.49+0.025=0.074
The Kappa statistic was calculated using the Kappa formula

$$\frac{\text{Observed agreement-expected agreement}}{\text{1-expected agreement}}$$

Kappa coefficient: (0.94-0.074)/ (1-0.074) = 0.866/0.926 = 0.93

Then, a Kappa calculation on the ratings was performed to determine the inter-rater reliability of the selection process. For Kappa results, values ≤ 0 indicate no agreement and 0.01–0.20 mean none to slight, 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement (McHugh, 2012). As the Kappa coefficient was 0.93, the level of agreement was almost perfect.

Appendix C: Jadad Scale

Dimension			**Sub Score**
Randomization	1. Was the study described as randomized (this includes the use of words such as randomly, random, and randomization)? = 1 point	Give 1 additional point if: For question 1, the method to generate the sequence of randomization was described and it was appropriate (table of random numbers, computer generated, etc.) Deduct 1 point if: For question 1, the method to generate the sequence of randomization was described and it was inappropriate (patients were allocated alternately, or according to date of birth, hospital number, etc.)	
Blinding	2. Was the study described as double blind? = 1 point	Give 1 additional point: If for question 2 the method of double blinding was described and it was appropriate (identical placebo, active placebo, dummy, etc.) Deduct 1 point: If for question 2 the study was described as double blind, but the method of blinding was inappropriate (e.g., comparison of tablet vs. injection with no double dummy)	
Withdrawals and dropouts	3. Was there a description of withdrawals and dropouts? = 1 point		
		TOTAL JADAD SCORE	

Note: Jadad Scale. Adapted from “from National Center for Biotechnology Bookshelf (https://www.ncbi.nlm.nih.gov/books/NBK56923/#jadad) Copyright n.d by NCBI Bookshelf ID NBK56923

Jadad Guidelines for Assessment (National Center for Biotechnology Information, n.d.)

1. Randomization

   A method to generate the sequence of randomization will be regarded as appropriate if it allowed each study participant to have the same chance of receiving each intervention and the investigators could not predict which treatment was next. Methods of allocation using date of birth, date of admission, hospital numbers, or alternation should be not regarded as appropriate.

2. Double blinding

   A study must be regarded as double blind if the word “double blind” is used. The method will be regarded as appropriate if it is stated that neither the person doing the assessments nor the study participant could identify the intervention being assessed, or if in the absence of such a statement the use of active placebos, identical placebos, or dummies is mentioned.

3. Withdrawals and dropouts

   Participants who were included in the study but did not complete the observation period or who were not included in the analysis must be described. The number and the reasons for withdrawal in each group must be stated. If there were no withdrawals, it should be stated in the article. If there is no statement on withdrawals, this item must be given no points.

Appendix D: Table 3.4 (data extraction)

Type 1 Diabetes										
Title	**Trial Numbers and Study Design**	**Authors/ Sponsors**	**Date and Duration**	**Population**	**Intervention**	**Comparison**	**Outcome**	**Researcher Rating for Inclusion**	**Independent Rater for Inclusion**	**Jadad Scale**
Adaptive Study of IL-2 Dose Frequency on Regulatory T Cells in Type 1 Diabetes (DILfrequency)	NCT02265809 Non-randomized Single group assignment Open-label	Cambridge University Hospitals NHS Foundation Trust	October 3, 2014 2 years	38 patients, aged 18 to 70 years Type 1 diabetes Duration of diabetes less than 60 months 36 finished the trial	Aldesleukin will be administered subcutaneously at varying doses and frequencies for a period of up to 98 days from the first administration depending on the treatment assignment. The maximum dose allowed is 0.6 X 10^6 IU/m2.	Placebo	Blood glucose, HbA1c, C-peptide, insulin use and autoantibody status	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 0 Total Score = 0
Dose Finding Study of Il-2 at Ultra-low Dose in Children With Recently Diagnosed Type 1 Diabetes (DFIL2-Child)	NCT01862120 Randomized Parallel Assignment Single blinding	Assistance Publique - Hôpitaux de Paris	June 27, 2013 4 years	7 Years to 14 Years	Drug: Dose D1 of interleukin-2Drug: placebo Drug: Dose D2 of Interleukin-2Drug: Dose D3 of interleukin-2	placebo	Define the lowest dose of rhIL-2 inducing TREGS in children with recently diagnosed type 1 diabetes.	Yes	Yes	Randomization = 2 Blinding = 1 Account of all patients =1 Total Score = 4
Dose-effect Relationship of Low-dose IL-2 in Type 1 Diabetes (DF-IL2)	NCT01353833 Phase 1 &2 Randomized Parallel assignment Double blind	Assistance Publique - Hôpitaux de Paris	May 16, 2011 9month study	18 to 50 years, 25 participants	After inclusion (Day0), the patient receives a 5-day course of IL-2 or placebo. Patients are randomized in 4 arms receiving either a placebo or IL-2 doses of 0,33 - 1 or 3 million UI/day. Laboratory follow-up of peripheral blood T cell subsets will be performed at D0 to D6 (daily), D15, D22 and D60 by immunophenotyping and transcriptomics. Tolerance will be evaluated at D0-6, D15, D22, and D60.	placebo	Kinetic parameters of Treg proportions variation within CD4+ T cells in peripheral blood	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Interleukin-2 Therapy of Autoimmunity in Diabetes (ITAD): a phase 2, multicenter, double-blind, randomized, placebo-controlled trial	Trial registration: EudraCT 2017-002126-20 Randomized Double-blind Not parallel	University of Oxford	June 2, 2019, 6 months	45 participants that included Children and adolescents with either type 1 or type 2 diabetes	A total of 45 participants will be randomized in a 2:1 ratio to receive either ultra-low dose IL-2 (aldesleukin), at a dose of 0.2 x 10 6 IU/m 2 twice-weekly, given subcutaneously, or placebo, for 6 months.	placebo	Safety and efficacy of different dose levels of aldesleukin on glucose levels and immune system	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Low-dose rhIL-2 in Patients With Recently-diagnosed Type 1 Diabetes (DIABIL-2)	NCT02411253 Randomized Quadruple masking, phase 2, ongoing Parallel assignment	Assistance Publique - Hôpitaux de Paris	June 2015 7 years	6 years to 35 years 141 patients	rhIL-2	placebo	AUC (T0-T120) of serum C-peptide, determined after a mixed meal	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Regulatory T Cells in Type 1 Diabetes Patients Treated With IL-2 (DILT1D)	NCT01827735 Phase 1 and Phase 2 open-label Not randomized	Cambridge University Hospitals NHS Foundat	March 2013 1 year	18 to 50 years 40 participants	Aldesleukin (Proleukin)	placebo	The primary endpoint is based upon the percentage of CD4+T regulatory	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients

	Single group assignment	ion Trust								= 1 Total Score = 1
Autologous Immunoregulatory Dendritic Cells for Type 1 Diabetes Therapy	NCT00445913 Phase1 NCT02354911 Interventional (Clinical Trial) Phase 2 Randomized Double blind Crossover assignment	Diavacs, Inc.	October, 2015 4 years	Age 12 to 35 New-onset type 1 diabetes randomized within 100 days of diagnosis 24 participants	Diabetes-suppressive dendritic cell vaccine	placebo	plasma c-peptide at 12 and 24 months	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Cellular Therapy for Type 1 Diabetes Using Mesenchymal Stem Cells	NCT04061746 Interventional (Clinical Trial) Randomized Parallel Assignment Quadruple blind	The Medical University of South Carolina, National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)	February, 2020 Ongoing	18 to 30 yrs, any sex 60 participants	Patients in Group A will receive a single MSCs infusion Patients in Group B will receive a single infusion of placebo	placebo	12 month Change in C-peptide area under the curve after a 2-hour MMTT [Time frame: 1 year (plus or minus 30 days) after infusion] Change in beta cell function	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
PolyTreg Immunotherapy in Islet Transplantation	NCT03444064 Non-randomized, open-label	University of Alberta	Feb 23, 2018 Ongoing	18 participants	Ex vivo Expanded Autologous CD4+CD127lo/-CD25+ Polyclonal Regulatory T cells	None	Adverse events, c-peptide level	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1
cePolyTregs in Islet Transplantation (cePolyTregs)	NCT05349591 Interventional (Clinical Trial) Non-randomized Parallel assignment Open-label	University of Alberta	May 15, 2022. Ongoing	18 to 68 years, 11 participants	The treatment group will receive cePolyTregs 2 weeks after islet transplantation as immunotherapy to improve islet survival and reduce the need for immunosuppression drugs.	Placebo	Stimulated C-peptide level	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1
Diabetes Autoimmunity Withdrawn In New Onset and In Established Patients (SUNRISE)	NCT03895437 Interventional (Clinical Trial) Phase 2 Randomized Parallel assignment Triple blind	Tolerion, Inc.	June 17, 2019 Ongoing	78 participants	Biological: TOL-3021Other: TOL-3021 Placebo	placebo	C-peptide level and HbA1c	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
DIABGAD - Trial to Preserve Insulin Secretion in Type 1 Diabetes Using GAD-Alum (Diamyd) in Combination With Vitamin D and Ibuprofen	NCT01785108 Interventional (Clinical Trial) Phase 2	F. Swedish Child Diabetes, S. The Research Council of South East and A. B. Diamyd Medical	Feb 7, 2013 4 years	60 participants 10 years to 18 years Diagnosed with type 1 diabetes within 4 months of screening	Biological: GAD-Alum (Diamyd) 20 µgBiological: GAD-Alum (Diamyd) 20 µg X 2Drug: Vitamin DDrug: Ibuprofen	placebo	C-peptide level and HbA1c	No	No	
Evaluate Safety of Adipose-Derived Mesenchymal Stem Cell Transplantation for	NCT05308836 Non-randomized	Vinmec Research Institute of Stem Cell and	October 4, 2021 Ongoing	10 participants, 5 years and older	Intravenousling (IV) AD-MSC in 10 patients with type 1 diabetes mellitus.	none	Safety, HbAIc level, c-peptide level	Yes	Yes	Randomization = 0 Blinding = 0

Type 1 Diabetes Treatment	Single group assignment, open-label Phase1	Gene Technology								Account of all patients = 1 Total Score = 1
MER3101: MAS-1 Adjuvanted Antigen-specific Immunotherapeutic for Prevention and Treatment of Type 1 Diabetes (MER3101)	NCT03624062 Phase 1, randomized double-blinded sequential	University of Colorado, Denver	August 31, 2020 Ongoing	28 participants, 18 to 45 years	MAS-1 adjuvanted Insulin B-chain	placebo	C peptide level, HbA1c level,	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Pilot Clinical Trial of Ustekinumab in Patients With New-onset T1D (UST1D)	NCT02117765 Phases 1 &2 Single Group Assignment, open label	University of British Columbia	March 2015, 2 years	20 participants, 18 to 35 years	Ustekinumab or Stelara	Placebo	Immunological endpoints, Insulin	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 0 Total Score = 0
Safety and Efficacy of CLBS03 in Adolescents With Recent Onset Type 1 Diabetes (The Sanford Project T-Rex Study)	NCT02691247 Phase 2, randomized, quadruple masking Participant, Care Provider, Investigator, Outcomes Assessor	Caladrius Biosciences, Inc.	Feb 2016, 4 years	113 participants, 8 to 17 years	Autologous Ex Vivo Expanded Polyclonal Regulatory T-cells) CLBS03 Low Dose	placebo	C-peptide, HbA1c	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Safety, Tolerability and Potential Efficacy of AVT001 in Patients With Type 1 Diabetes	NCT03895996, Randomized, parallel, quadruple	Avotres Inc.	June 20, 2019 Ongoing	24 participants, 16 years and older	autologous dendritic cell therapy AVT001	placebo	Safety, Treg cells, HbA1c	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
A Study to Assess the Safety and Tolerability of Different Doses of AG019 Administered Alone or in Combination With Teplizumab in Participants With Recently Diagnosed Type 1 Diabetes Mellitus (T1D)	NCT03751007, Phase 1b – open label, phase 2a – randomized, double blind	Precigen Actobio T1D, LLC	October 27, 2018, 3 years	42 participants, This study consists of 2 phases: Phase 1b: this open-label part of the study will investigate the safety and tolerability of 2 different doses of AG019 in 2 age groups (18-40 years of age and 12-17 years of age). Phase 2a: this randomized, double-blind part of the study will investigate the safety	AG019 (gene transference, IL10 expression modulators), Teplizumab (anti-CD3 monoclonal antibody)	Placebo	Safety and tolerability of AG019 and Teplizumab	Yes	Yes	Phase 1b: Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1 Phase 2a: Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5

				and tolerability of AG019, in association with teplizumab, in 2 age groups (18-40 years of age and 12-17 years of age)						
T1DM Immunotherapy Using CD4+CD127lo/-CD25+ Polyclonal Tregs (Treg)	NCT01210664 Phase 1 Interventional, Open label	University of California, San Francisco	November, 2010, 7 years	18 to 45 years 16 participants with type 1 diabetes	Biological: Ex vivo Expanded Human Autologous Polyclonal Regulatory T Cells	placebo	Adverse events, c peptide, HbA1C	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1
T1DM Immunotherapy Using Polyclonal Tregs + IL-2 (TILT)	NCT02772679 Phase 1 interventional, open label	Yale university, University of California, San Francisco	May 13, 2016 5 Years	18 to 45 years 16 participants with type 1 diabetes	Biological: PolyTregs+IL-2	placebo	Adverse events, c peptide, survival of tregs, HbAIc	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1
Open-label Investigation of the Safety and Efficacy of DIABECELL in Patients with Type 1 Diabetes Mellitus	NCT00940173 Open-label, Non-randomized, Single group assignment	Living Cell Technologies	July 2009, 4 years	14 participants, 18 to 65 years	DIABECELL	None	Function, efficacy and safety	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1
Open-label Investigation of the Safety and Efficacy of DIABECELL in Patients With Type 1 Diabetes Mellitus	NCT01736228 Open-label, Non-randomized, Single group assignment	Diatranz Otsuka, Ltd	November 2012, 2 years	8 participants, 18 to 65 years	DIABECELL	None	Function, efficacy and safety	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1
Open-label Investigation of the Safety and Effectiveness of DIABECELL® in Patients With Type 1 Diabetes Mellitus	NCT01736229	Diatranz Otsuka, Ltd	August 2011, 3 years	14 participants, 18 to 65 years	DIABECELL	None	Function, efficacy and safety	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1
Safety and Efficacy Study of Islets Xenotransplantation	NCT03162237 Randomized, open-label	Hunan Xeno-life Science Ltd	July 13, 2013 5 years	20 participants	Porcine islets Autologous Treg	None	Blood glucose level, c-peptide level, hemoglobin A1C	Yes	Yes	Randomization = 2 Blinding = 0 Account of all patients = 1 Total Score = 3
Teplizumab for Prevention of Type 1 Diabetes In Relatives "At-Risk"	NCT01030861 Interventional, randomized, double blind	National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)	August 2010, 9 years	76 participants	anti-CD3 monoclonal antibody, teplizumab	None	Rate of diabetes per year	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score =5
Type 2 Diabetes										

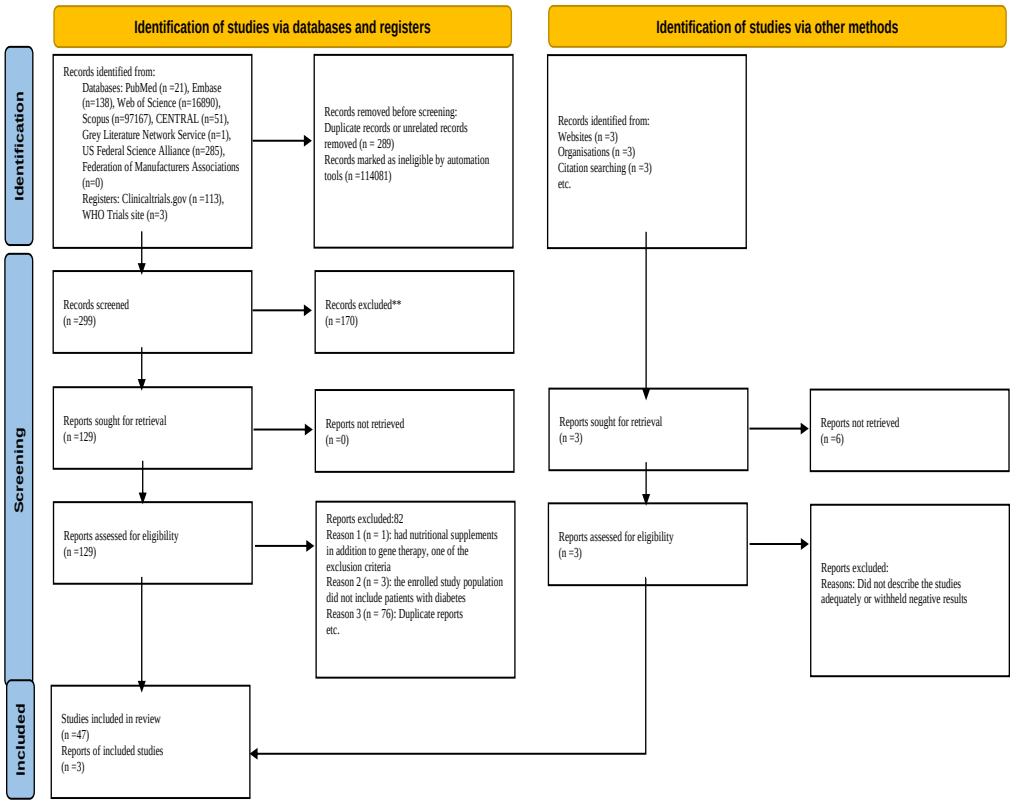
Title	Trial Numbers and Study Design	Authors/ Sponsors	Date and Duration	Population	Intervention	Comparison	Outcome	Researcher Rating for Inclusion	Independent Rater for Inclusion	Jadad Scale
Safety and Tolerability After Four Weeks of Treatment with AZD1656 in Patients with Type 2 Diabetes	NCT00856908 randomized, double-blind, placebo-controlled	AstraZeneca	Feb 2009, 6 months	224, 35 to 75 years old	Drug: AZD1656	Placebo	Safety, tolerability, Glucose and HbAIc level	Yes	Yes	Randomization =2 Blinding=2 Account of all patients =1 Total Score =5
A Study to Evaluate P-Glucose, Safety, and Tolerability After Oral Single Dosing of AZD6370 in Type 2 Diabetic Patients	NCT00690287 randomized, single-blind, placebo-controlled, crossover assignment	AstraZeneca	Feb 2008 6 months	24 participants, 30 to 65 years	AZD-6370	Placebo	Dose-finding study	Yes	Yes	Randomization =2 Blinding=1 Account of all patients =1 Total Score =4
AZD1656 in Transplantation With Diabetes tO PromoTe Immune TOleraNce (ADOPTION) Type 2	NCT05216172 Interventional a single-site, placebo-controlled, double-blind randomized clinical trial Phase 2	AstraZeneca	January 21, 2020 to September 2022 Ongoing	50 patients, 18 years and older renal transplant patients with Type 2 diabetes	Drug: AZD1656	Placebo	Change in mean peripheral Treg cell number between baseline and 3 months measured glycemic control: HbA1c	Yes	Yes	Randomization =2 Blinding=2 Account of all patients =1 Total Score =5
Long term Follow-up of Subjects with Diabetes 2 Type Treatment With ex Vivo Gene Therapy (AUB001)	NCT04642911 10 year follow up of intervention	Ukraine Association of Biobank	October 15,2020 10 years	91 participants Type 2 diabetes Child, adult, older adult	10 years follow up of (Mesenchymal Stem Cell)	None	Overall survival of subject with diabetes 2 type with gene therapy drug product (Mesenchymal Stem Cell)	Yes	Yes	Randomization =0 Blinding=0 Account of all patients =1 Total Score =1
p53 Gene Therapy in Treatment of Diabetes Concurrent with Hepatocellular Carcinoma	NCT02561546 Interventional clinical trial Phase 2 This is an open-labeled, randomized, active-controlled phase 2 study.	Shenzhen SiBiono GeneTech Co.,Ltd	December 2015, 2 years	40 participants with type 2 and Hepatocellular carcinoma	Drug: p53 gene therapy Other names: recombinant adenoviral human p53 gene therapy Drug: Trans-catheter embolization	Drug: p53 gene therapy Other names: recombinant adenoviral human p53 gene therapy Drug: Trans-catheter embolization	Fasting plasma glucose, HbAIC	Yes	Yes	Randomization =2 Blinding=0 Account of all patients =1 Total Score =3
Outcomes of Expanded Autologous Bone Marrow-derived Mesenchymal Stem Cells Therapy in Type II	NCT03343782 Phases 1 &2, open-label single group assignment	Vinmec Research Institute of Stem Cell and Gene Technology	November 1, 2017 2 years	18 years older,30 participants	Combination Product: Expanded autologous bone marrow-derived mesenchymal stem cell	Placebo	Insulin dose, Adverse events, HbA1c	Yes	Yes	Randomization =0 Blinding=0 Account of all patients =1 Total Score =1

Diabetes (ASD2)										
Diabetes Related Complications										
Title	**Trial Numbers and Study Design**	**Authors/ Sponsors**	**Date and Duration**	**Population**	**Intervention**	**Comparison**	**Outcome**	**Researcher Rating for Inclusion**	**Independent Rater for Inclusion**	**Jadad Scale**
VEGF gene transfer for diabetic neuropathy	NCT00056290 Interventional (Clinical Trial) phase 1 and 2 Randomized Parallel assignment Triple blinded	Losordo, Douglas, M.D.	December 2002 6 years	60 participants, 21 Years and older (Adult, Older Adult) Type 1 and 2 diabetes and peripheral neuropathy	Biological: VEGF	Placebo	Safety and growth of new blood vessels	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Safety and Efficacy Study for the Treatment of Painful Diabetic Neuropathy	NCT01475786 Double blind (participant, investigator) Placebo controlled Randomized Parallel assignment	Helixmith Co., Ltd.	August 2012, 2 years	104 patients with diabetic neuropathy	VM 202	Placebo	Mean symptom score, any adverse events	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 0 Total Score = 4
Gene Therapy for Painful Diabetic Neuropathy	NCT01002235 Non-randomized Sequential, Open label	Helixmith Co., Ltd.	January 30, 2010 2 years	18 to 75 years, 12 participants	VM 202	none	Safety and tolerability	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 0 Total Score = 1
Phase 3 Gene Therapy for Painful Diabetic Neuropathy	NCT02427464 Interventional, Randomized, quadruple blinding, placebo controlled parallel assignment	Helixmith Co., Ltd.	January 30, 2016 3 years	18 to 75 years, 507 participants	VM 202	placebo	Safety, difference in pain score	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Extension of Phase 3 Gene Therapy for Painful Diabetic Neuropathy	NCT04055090 Follow up of Double-blind Randomized Placebo-controlled	Helixmith Co., Ltd.	January 30, 2019 6 months	18 to 75 years, 101 participants	VM 202	placebo	Safety, difference in pain score	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Treatment with intramuscular vascular endothelial growth factor gene compared with placebo for patients with diabetes mellitus and critical limb ischemia: a double-blind randomized trial	Double-blind placebo controlled study Randomized	Kusumanto et al.	2006, 100 days	54 adult diabetic patients	phVEGF165	placebo	Safety, effectiveness, amputation rate at 100 days	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Study of HGF Via Plasmid Vector to Improve Perfusion in Critical Limb Ischemia	NCT00060892 Randomized, double blind, placebo controlled	AnGes USA, Inc.	April 2003 4 years	104 participants	HGF plasmid	placebo	Dose finding study to improve	No	Yes	
Study of Hepatocyte Growth Factor (HGF) Via Plasmid Vector to Improve Perfusion in	NCT00189540 Double blind randomized placebo controlled	AnGes USA, Inc.	Sept 19, 2005 3 years	27 participants 40 years and older	HGF plasmid	placebo	Would healing, percentage of participants where all	No	Yes	

Critical Limb Ischemia Patients With Peripheral Ischemic Ulcers							ulcers healed.			
Safety and efficacy of patient specific intramuscular injection of HGF plasmid gene therapy on limb perfusion and wound healing in patients with ischemic lower extremity ulceration: results of the HGF-0205 trial	Results from the study above	AnGes USA, Inc.	Sept 19, 2005 3 years	27 participants 40 years and older	HGF plasmid	placebo	Would healing, percentage of participants where all ulcers healed.	No	Yes	
A phase I clinical study of naked DNA expressing two isoforms of hepatocyte growth factor to treat patients with critical limb ischemia	Open-label, non- placebo controlled, dose-escalation, single-center study	Gu et al,	November 2008 One year	21 patients, 20-80 years old	NL003 (pCK-HGF-X7)	None	Safety, tolerability and efficacy	Yes	Yes	Randomization = 1 Blinding = 1 Account of all patients = 1 Total Score = 1
A Randomized, Double-Blind, Placebo-Controlled Phase II Study of Hepatocyte Growth Factor in the Treatment of Critical Limb Ischemia	Randomized Double-Blind, Placebo-Controlled Phase II	Gu et al.	March 2012 2 years	200 patients, 50 years and older	NL003 (pCK-HGF-X7)	placebo	Pain, proportion of ulcer healing	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Clinical Safety and Preliminary Efficacy of Plasmid pUDK-HGF Expressing Human Hepatocyte Growth Factor (HGF) in Patients with Critical Limb Ischemia	Prospective, open label, dose escalation, single center study	Gu et al.	2012 One year	21 patients, 20-80 years old	pUDK-HGF	None	Safety, tolerability and efficacy	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1
Insights on the role of diabetes and geographic variation in patients with critical limb ischemia Same studyEfficacy and Safety of XRP0038/NV1FGF in Critical Limb Ischemia Patients With Skin Lesions (TAMARIS)	NCT00566657 Randomized, double blind Parallel assignment+	Belle et al Sanofi	November, 2007 5 years	525 participants Recruited from 170 cities worldwide 60 years old and older	riferminogene pecaplasmid NV1FGF XRP0038	placebo	Variations by region of origin and status	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
Double VEGF/HGF Gene Therapy in Critical Limb Ischemia Complicated by Diabetes Mellitus	Randomized double blind	Barc et al.	90 days	28 patients With critical limb ischemia Complicated by diabetes	(VEGF/HGF) gene therapy	None	Rest pain score	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1

										Total Score = 5
Phase I study of multi-gene cell therapy in patients with peripheral artery disease	NCT00390767 Open label Single group assignment No randomization	MultiGene Vascular Systems Ltd	October 20, 2006 Ongoing	12 participants	MultiGeneAngio/MGA	Placebo	Safety of MGA, improvement of PAD systems.	Yes	Yes	Randomization = 0 Blinding = 0 Account of all patients = 1 Total Score = 1
SDF-1 plasmid treatment for patients with peripheral artery disease (STOP-PAD): Randomized, double-blind, placebo-controlled clinical trial	NCT02544204 Phase 2 Randomized Parallel assignment Double blind	Juventas Therapeutics, Inc.	November 2015, 2 years	120 participants 18 years and older	JVS-100, SDF1 plasmid	Placebo	Wound healing, adverse effects	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
ADVM-022 Intravitreal Gene Therapy for DME (INFINITY)	NCT04418427 Phase 2 Randomized, open label	Adverum Biotechnologies, Inc.	May 28, 2020 Ongoing	36 patients with with either macular edema	ADVM-022 (AAV.7m8-aflibercept)	baseline	improvement in diabetic retinopathy severity scale (DRSS) scores	Yes	Yes	Randomization = 2 Blinding = 0 Account of all patients = 1 Total Score = 3
Safety and Efficacy Study of rAAV.sFlt-1 in Patients With Exudative Age-Related Macular Degeneration (AMD)	NCT01494805 Randomized Parallel assignment Single blind	Lions Eye Institute Adverum Biotechnologies, Inc. 2 phases and 3 year follow up	December 2011, 6 years,	40 participants 55 years or older	Biological: rAAV.sFlt-1Other	Control (ranibizumab alone)	Ocular and systematic safety, adverse events, visual acuity	Yes	Yes	Randomization = 2 Blinding = 1 Account of all patients = 1 Total Score = 4
Angiogenic gene therapy does not cause retinal pathology	Randomized, double-blind Placebo-controlled	Prokosch et all	2014, 12 months	152 patients with critical limb ischemia	NV1FGF	placebo	If this treatment caused retinal pathology	Yes	Yes	Randomization = 2 Blinding = 2 Account of all patients = 1 Total Score = 5
RGX-314 Gene Therapy Administered in the Suprachoroidal Space for Participants With Diabetic Retinopathy (DR) Without Center Involved-Diabetic Macular Edema (CI-DME) (ALTITUDE)	NCT04567550 Phase 2 Randomized Parallel assignment Open label	REGENXBIO Inc.	November,2020 Ongoing	60 participants, Ages 25 and 89	Genetic: RGX-314 Dose	1Genetic: RGX-314 Dose 2	Effect of RGX-314 based on dose and time	Yes	Yes	Randomization = 2 Blinding = 0 Account of all patients = 1 Total Score = 3

Appendix E: PRISMA Flow Chart 2020



Note: PRISMA flow diagram for new systematic reviews which included searches of databases, registers, and other sources. Adapted from "PRISMA Transparent Reporting of Systematic Reviews and Meta-Analysis", from PRISMA (http://prisma-statement.org/prismastatement/flowdiagram.aspx). Copyright 2020 by PRISMA.