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Review

Charting the next century of insulin replacement with cell and gene therapies

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SUMMARY

The discovery of insulin a century ago changed the lives of millions of individuals suffering from diabetes, paving the way for long-term survival. While the availability of recombinant insulin for hormone replacement therapy has served extremely well to help control blood glucose in diabetes, there remains significant room for further improvements for an ultimate "cure" for diabetes patients. In this review, we celebrate the 100th anniversary of the discovery of insulin and consolidate the key milestones and advances in the development of recombinant human insulin. We then summarize recent and current technological developments in terms of insulin gene- and cell-replacement therapies that are promising in greater therapeutic potential. We envision that the next era of insulin replacement therapies will effectively treat diabetes and serve our patients even better for the next century to come.

INTRODUCTION

In 2021, we celebrate the 100th birthday of insulin, whose discovery has since improved the lives of millions of people suffering from diabetes mellitus. The dysfunction and death of insulin-producing β cells is ultimately the central pathogenesis leading to diabetes. The degree of β cell dysfunction could directly affect the age of onset of diabetes. As such, diabetes (excluding the transitory nature of gestational diabetes) can be broadly categorized into monogenic, type 1 diabetes (T1D) and type 2 Diabetes (T2D). The various forms of diabetes vary in their underlying molecular mechanisms leading to their manifestation, but all of them present with a common clinical outcome—hyperglycemia. T1D occurs as a consequence of autoimmune-mediated destruction of insulin-producing pancreatic β cells, causing patients to have insufficient insulin in their body. The most common form of diabetes, T2D, is associated with eventual β cell exhaustion and progressive deterioration of glycemic control in patients, accompanied by insulin resistance and/or obesity.

The first few decades of insulin therapy since its discovery in 1921 focused on generating large-scale supplies of high-purity insulin with improved pharmacokinetics to meet the increasing demand worldwide. The key strategy adopted was to create a "one-for-all" treatment for diabetes patients. With a better understanding of the mechanisms underlying the various types of diabetes over the years, the notion of multiple routes to β cell failure became clear.[1] The current focus for insulin therapy is increasingly on safety, convenience, and a more targeted manner to cater to the heterogeneity across the various classes of diabetes.[2–4] The emergence of numerous new diabetes drug classes has occurred in the last decades, but insulin remains the only option for diabetes treatment in patients who are no longer able to produce (sufficient) insulin autonomously. Banting prophetically commented that "Insulin is a treatment, but not a cure"[5]. Side effects such as risks of hypoglycemia

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and weight gain certainly persist. In this review, we present the key milestones of insulin therapies over the last century, providing a summary on the current insulin analogs, the various routes of insulin delivery, the progress on insulin gene- and cell-replacement therapy, and, finally, a discussion on the priorities to chart the next century for insulin-replacement therapy.

Development of insulin therapy: From then until now

Physiological insulin secretion consists of a constant low basal secretion and a markedly increased prandial secretion that is correlated with food/glucose intake. The basal insulin secretion limits lipolysis and hepatic glucose production at fasting state, keeping basal blood glucose levels consistent. However, prandial insulin secretion stimulates the utilization of glucose by peripheral tissues, thereby regulating hyperglycemia after a meal.[6] Prandial insulin is released into the bloodstream in a biphasic manner in response to an acute elevation of glucose. The first-phase release is rapid (<2 min), triggering a sharp peak that subsides within 10 min. The second phase of insulin secretion is triggered following a prolonged exposure to glucose until normoglycemia is restored.[7] To achieve optimal glycemic control, insulin secretion and clearance are highly regulated, and the insulin receptors mediate insulin uptake and degradation. Moreover, plasma insulin is short-lived (4–6 min), as would be expected for rapid response to changes in blood glucose.[8] In the subsequent sections, we discuss the development of insulin formulations that attempt to mimic the kinetics of the complex endogenous insulin secretion and clearance patterns in the past few decades.

The rise of recombinant human insulin

Insulin was obtained from animal pancreatic tissues to treat diabetes before the availability of recombinant DNA (rDNA) technology. Often, the yield was low and potency was variable (~25%). Toward the end of 1922, using isoelectric precipitation, animal insulin was able to be produced at higher purity and stability to match the clinical demand.[9] As individuals living with diabetes require multiple daily insulin injections, the need for insulin with a long action profile was desirable. In 1930s, the addition of protamine or zinc to insulin preparations prolonged its duration of action significantly, reducing the number of daily injections required.[10] These discoveries marked the first step toward the development of insulin with different pharmacokinetic and pharmacodynamic profiles.

The primary sequence and disulfide configuration of sheep insulin was elucidated in 1954[11]. Decades later, in 1978, the first recombinant human insulin was prepared by combining the insulin A and B chains expressed in *Escherichia coli*.[10] In 1982, large-scale production of recombinant human insulin of high purity using fermentation of microorganisms (bacteria and yeast) became available.[12,13] This was a significant milestone as it offered a cost-effective means of producing an unlimited supply of insulin with high purity as compared to crude animal pancreatic extracts.

The Diabetes Control and Complications Trial (DCCT) using intensive insulin therapy (INT) was carried out from 1983 to 1993 to address the relationship between the degree of glycemic control and complication prevalence.[14] Its findings definitively demonstrated that the study group under INT designed to achieve near-normal glycemia displayed a lower prevalence of complications (that affect the eyes, kidneys, peripheral and autonomic nervous systems, and cardiovascular function).[14] The major adverse effects associated with INT, whereby regular insulin was administered before meals and long-acting insulin was administered at bedtime, was the elevated risk of severe hypoglycemia and increased obesity. Nonetheless, the benefits of INT

still outweigh its adverse effects, establishing INT as the standard of care for T1D management. The development of insulin formulations that closely mimicked the kinetics of insulin secreted by β cells became the next quest.

Rapid-acting insulin

Regular insulin (animal insulin, recombinant human insulin) adopts the native structure of endogenous insulin and is often administered to T1D patients to replace prandial insulin. However, when regular insulin is injected subcutaneously, the rate of hexamer dissociation into absorbable monomeric form is rate limiting. The injection site acts as a depot for the dissociation of hexamer to occur and often takes 2–4 h for the peak effect.[15] Therefore, to achieve post-prandial glycemic control, insulin should be injected at least 30 min before meals. Besides the late onset of action, regular insulin also acts longer in a dosage-dependent manner.

In pancreatic β cells, native mature insulin is stored in secretory granules as compacted hexamers coordinated with the Zn^{2+} ion. The presence of Zn^{2+} allows for chelation with serum albumin upon insulin exocytosis, quickly releasing insulin from its hexameric form.[16] To shorten the duration of onset and action, rapid-acting insulin analogs were introduced in the 1990s. Insulin lispro, aspart, and glulisine adopt similar manipulations on the primary structure of the insulin B chain to achieve enhanced solubility and rapid dissociation of insulin hexamers for faster absorption. Lispro has amino acid residues at positions 28 and 29 (proline and lysine) swapped, aspart has its non-charged proline at position 28 substituted with polar lysine, while glulisine has lysine and glutamic acid (highly polar) substituting for asparagine and lysine at positions 3 and 29, respectively[17] (Figure 1A). These modifications increased their solubility, reducing the onset of action to <15 min and a shorter duration of action (3–5 h) in lispro, aspart, and glulisine,[17] providing superior control of prandial glucose over regular insulin (Table 1).

Ultra-rapid-acting insulin analogs were introduced in 2020, further shortening the duration of onset to <5 min. Fast-acting aspart consists of two additional excipients, niacinamide and L-arginine, further accelerating the rate of absorption.[23] Ultra-rapid-acting lispro was also approved in 2020, in which two excipients, trepostinil and sodium citrate, were added to facilitate insulin absorption. Trepostinil is a prostacyclin analog that promotes local vasodilation, while sodium citrate enhances the rate of absorption by increasing local vascular permeability.[24] These ultra-rapid-acting insulin formulations provided better post-prandial glycemic control over the rapid-acting insulin analogs, when administered before or within 20 min of a meal.[24,25] The strategies adopted for rapid-acting insulin analogs are depicted in Figure 1B.

This shorter duration of onset means that insulin analogs could be administered after meals, enhancing patient convenience and minimizing the risks associated with hypoglycemia when a scheduled meal is not eaten. While rapid-acting insulin analogs are superior to regular insulin in terms of the rapid onset, they are still less than ideal. Often, insulin is prescribed in a pre-determined dosage for each patient without consideration of the meal size and glycemic index of the food ingested. This means that patients are still subjected to suboptimal glycemic control from under- or over-dosing of insulin.

Long-acting insulin

Long-acting insulin attempts to recreate a peakless, reproducible pharmacodynamic profile that mimics the low and constant basal insulin in healthy individuals in between meals and through the night (Figure 1B). The addition of Zn or protamine

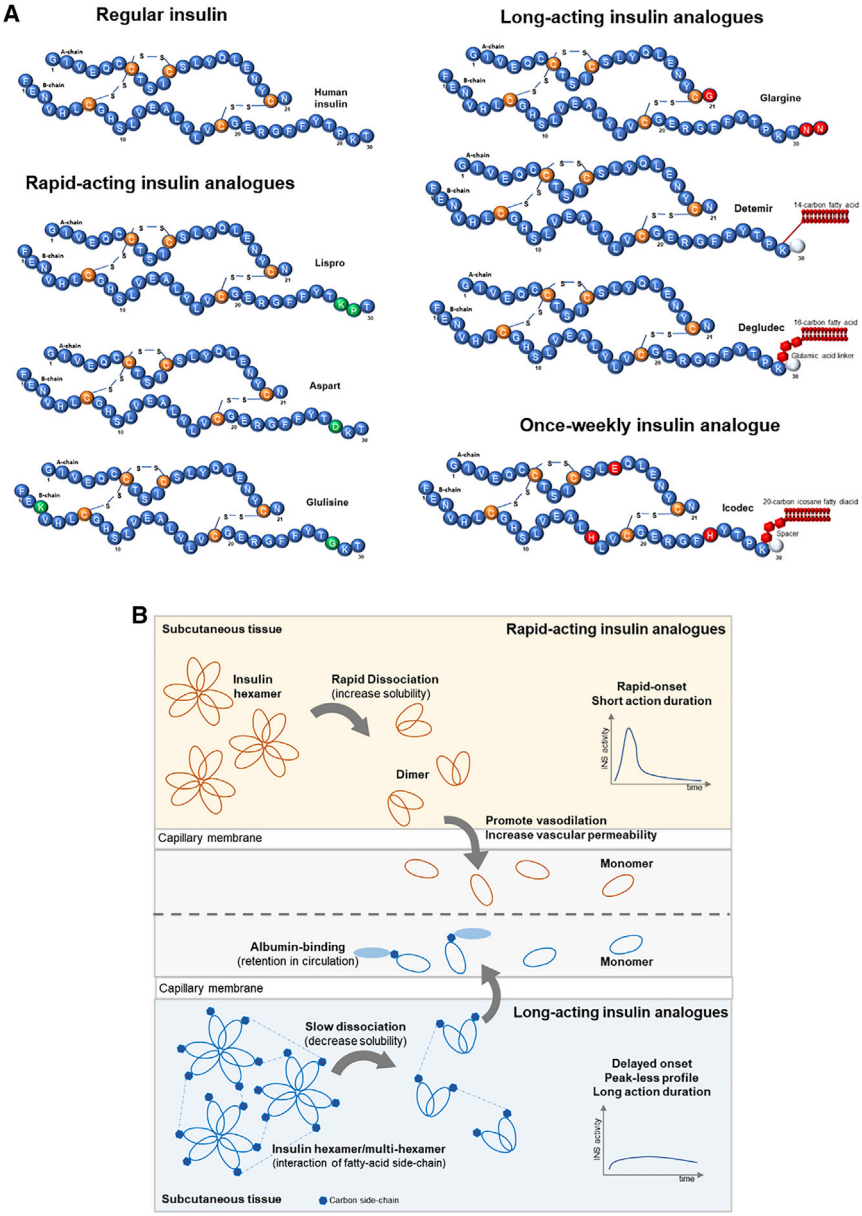


Figure 1. Amino acid structural arrangement of insulin analogs and their respective action profiles

(A) Schematic of human insulin showing amino acid arrangement of the A and B chains linked by disulfide bonds. Modifications to amino acid sequences in rapid-acting insulin analogs lispro, aspart, and glulisine are in green. Modifications to amino acid sequences in long-acting insulin analogs glargine, determir, and degludec are in red. Once-weekly insulin analog icodec is undergoing clinical trial.

(B) Schematic of rapid-acting or long-acting insulin analog dissociation at the site of injection. The strategies to accelerate absorption or facilitate protraction for rapid-acting and long-acting insulin analogs are illustrated, respectively.

(Zn-based insulin and neutral protamine Hagedorn [NPH] insulin) to regular insulin created a heterogenous suspension that prolonged the insulin action profile by acting as a depot at the site of administration. In subcutaneous tissue, the rate of insulin hexamers being released from the depot is highly variable. NPH insulin has a

Table 1. List of recombinant human insulins that are clinically available (unless otherwise stated)

Insulin analog	Brand	Onset of action	Duration of action
Prandial insulin			
Regular insulin	HumulinR NovolinR InsumanR	30–60 min	5–8 h
Lispro	Humalog Admelog	15–30 min	3–5 h
Aspart	Novolog	15 min	3–5 h
Glulisine	Apidra	12–30 min	3–5 h
Faster-prandial insulin			
Fast-acting aspart	Fiasp	3–4 min	3–5 h
Ultra-rapid lispro	URLi	3–4 min	3–5 h
Basal insulin			
NPH insulin	HumulinN NovolinN	1–2 h	14–24 h Highly variable
Glargine	Lantus Basaglar Toujeo (U-300)	NA	24 h
Determir	Levemir	NA	7.6–24 h
Deglutec	Tresiba	NA	42 h
Icodec[18]	–	NA	196 h (1 week)
Pre-mixed insulin			
NPH/regular insulin (70/30)	Humulin 70/30 Novolin 70/30	same as the respective parent analogs	
NPH/regular insulin (50/50)	Humulin 50/50	same as the respective parent analogs	
Lispro-protamine/lispro (75/25)	Humalog 75/25	same as the respective parent analogs	
Lispro-protamine/lispro (50/50)	Humalog 50/50	same as the respective parent analogs	
Aspart-protamine/aspart (70/30)	Novolog 70/30	same as the respective parent analogs	
Aspart/degludec	Ryzodeg	same as the respective parent analogs	
Fixed-ratio combination insulin			
Degludec/GLP-1RA (liraglutide)	IDegLira[19,20]	NA	same as the parent analog
Glargine/GLP-1RA (lixisenatide)	iGlarLixi[21,20]	NA	same as the parent analog
ADO09 (pramlintide/A21G)	[22]	NA	NA

Time-action profiles taken from Hirsch et al.,[15] unless otherwise stated. Prandial and basal insulin analogs are available at higher concentrations (not included in the table). Icodec and ADO09 are in clinical trials. GLP-1 RA, glucagon-like peptide 1 receptor agonist; NPH, neutral protamine Hagedorn.

delayed onset of action (2.5–3 h), followed by a peak effect at 5–7 h and a prolonged duration of up to 13 h.[26] In some T1D patients, the peak effect and short duration action profiles do not translate well to a clinically satisfactory basal insulin effect. Hence, they must be administered more than twice daily.[27,28] Besides the high variability in action profiles, some human insulin formulations show a pronounced peak effect, which could pose a risk of nocturnal hypoglycemia. For instance, first introduced in 1950s and used for decades as basal insulin replacement (IR), lente insulin (Zn-based formulation) demonstrated peak effects and less predictable pharmacodynamics, leading to its eventual discontinuation along with ultralente insulin.[27,29]

To achieve clinically satisfactory basal IR, the desired outcomes include (1) peakless action profile of plasma insulin post-administration, (2) enhanced day-to-day reproducibility, and (3) elimination of nocturnal hypoglycemia. Applying the same principle adopted in the 1920s, the first commercially available long-acting insulin analog, glargine, was developed by right-shifting the isoelectric point[30] . The molecular modifications to the amino acids gave rise to a relatively peakless action profile with an extended duration of 24 h,[31] and translated to less nocturnal hypoglycemia as

compared to NPH in patients.[32] Detemir was then developed via modification to the insulin B chain, removing threonine flanking the C terminus and conjugating the lysine residue to a 14-carbon long-chain fatty acid. The cross-interaction of fatty acid side chains resulted in hexamer-dimerization and albumin binding, slowing its absorption into peripheral tissues and therefore improving pharmacodynamic consistency.[33] Degludec is currently the longest-acting insulin analog commercially available, with an action duration exceeding 40 h. On top of the same molecular modifications to threonine and lysine as detemir, degludec consists of a 16-carbon fatty acid chain conjugated to the lysine residue through a hydrophilic glutamic acid linker (Figure 1A). The gradual dissociation of degludec monomers results in a stable and peakless pharmacokinetic profile.[34] The protraction strategies adopted for long-acting insulin analogs are depicted in Figure 1B. The insulin analogs are also available at higher concentrations to provide a longer duration of action.

Two recent clinical trials (NCT03951805 and NCT03922750) evaluated icodec as a once-weekly basal insulin analog.[18,35] Three amino acid substitutions and a 20-carbon fatty diacid were introduced to enhance stability along with strong interaction with albumin for prolonged action in icodec (Figure 1). Icodec appeared to be well tolerated when transiting from daily basal insulin in T2D patients, demonstrating effective glycemic control with no elevated risks of hypoglycemia when compared with insulin glargine.[18] Once approved, once-weekly insulin administration may further reduce the frequency of injections because of its additional convenience.

Pre-mixed insulin formulations

The rationale of pre-mixed formulations is to offer a simpler insulin regimen to patients through combining rapid- and long-acting insulin to cater to daily requirements. Pre-mixed insulins consisting of various ratios of rapid- and long-/intermediate-acting formulations are available on the market, mixing NPH insulin with regular insulin (70/30, or 50/50, respectively).[15,36] The pre-mixed insulins retain their respective pharmacokinetic profiles and need to be administered at least 30 min before a meal.[15,36]

While the variability of action profiles from NPH insulin remains a concern in pre-mixed formulations, insulin analog mixtures do demonstrate a superior post-prandial glucose control over human insulin mixtures, mainly attributed to the accelerated onset of action from rapid-acting analogs following subcutaneous administration.[17] The initial strategy adopted in the formulation of an insulin analog mixture is to combine rapid-acting analogs with protamine to prolong the duration of action. Insulin lispro and aspart premixes with their respective protamine suspensions are available at various mixture ratios (Table 1). The only pre-mixed insulin analogs without addition of protamine were first introduced in 2015, consisting of the rapid-acting insulin aspart and the long-acting degludec, marketed as Ryzodeg.[15] Besides the more predictable action profiles in analog-based pre-mixed formulations, the shorter onset of action translates to enhanced patient convenience by allowing administration shortly after a meal and obviates the need to resuspend the insulin before the injection.

Combination insulin formulations

Glucagon-like peptide 1 (GLP-1) is a hormone secreted by enteroendocrine cells of the intestine to regulate glycemia via potentiating insulin secretion in β cells while inhibiting glucagon secretion in pancreatic α cells. In addition, GLP-1 reduces the rate of gastric emptying, thereby lowering food intake.[37] Therefore, GLP-1 receptor agonists (RAs) have been developed and are shown to be effective in glucose lowering when prescribed to individuals with T2D.[38] The first two combinations of insulin with GLP-1 RAs that are commercially available comprise either insulin

degludec or glargine with a GLP-1 RA analog (liraglutide or lixisenatide, respectively) in a fixed ratio[19–21,39] (Table 1).

With its Phase II trial (NCT03981627) completed recently, ADO09 is a co-formulation of pramlintide and the rapid-acting A21G human insulin analog (the metabolite of glargine). Pramlintide is a US Food and Drug Administration (FDA)-approved amylin analog for use in conjunction with prandial insulin by individuals with T1D or T2D. Similar to the endocrine hormone amylin, pramlintide lowers serum glucose via reducing glucagon secretion, rate of gastric emptying, and food intake.[40] Findings from the clinical trial noted a slight increase in hypoglycemic episodes in ADO09 when compared with aspart, but there was no severe hypoglycemia observed. Subjects on ADO09 also required a lower prandial insulin dosage and had some weight loss (a mean of 1.6 kg over 24 days), whereas subjects taking aspart in the same study gained a mean of 0.4 kg of body weight.[22] The observations were associated with decreased appetite in subjects taking ADO09,[22] which is one of the physiological effects of amylin.

Next-generation insulin (the glucose-responsive insulin)

Endogenous insulin secretion by pancreatic β cells is regulated in a glucose-dependent manner that prevents the occurrence of hypoglycemia. This unique nature of secretion profile is lacking in biosynthetic insulin. Insulin is classified as a drug with a narrow therapeutic index, bearing the life-threatening risk of hypoglycemia when used outside its therapeutic dosage. The effort made on the development of a diabetes therapy with enhanced safety and convenience continues, with increasing efforts seen in the fabrication of glucose-responsive insulin (GRI).[41–43] The GRI technology aims to mimic the key insulin-secreting properties of β cells: (1) plasma glucose dependency and (2) glycemic control without risk of hypoglycemia. Among the GRIs reported, MK-2640 demonstrated glucose-responsiveness in two preclinical animal models while preventing hypoglycemia by initiating its clearance in a glucose-dependent manner.[41,43] MK-2640 is made by conjugating saccharides to regular insulin with the capacity to bind to the mannose receptor C-type 1 (MR, whose native function involves facilitating the clearance of glycated proteins) while retaining the capacity to bind to the insulin receptor.[41] Increased binding of MK-2640 to MR is observed *in vitro* when glucose concentration is low and vice versa, leading to MR-dependent lysosomal clearance of MK-2640, hence reducing the risk of hypoglycemia.[41] However, despite desirable outcomes obtained through preclinical studies, MK-2640 failed to achieve clinical proof-of-concept. This highlights the need to improve the predictive value of experimental results obtained in animal models.[44]

Strategies for insulin administration

The strategies for insulin administration have been a subject of intense investigation, with the aim of achieving enhanced glycemic control, improving patient convenience and adherence. New devices and systems for insulin administration have multiplied in the last 3 decades. Subcutaneous insulin injections are still the most widely used. While most patients still use insulin syringes, many also use disposable pens that are prefilled with insulin or pens that are preloaded with replaceable cartridges. These pens offer a simple, safe, and convenient method, and greatly overcomes the resistance and fear of using insulin syringes and needles. They also result in better glycemic outcomes when compared with the use of insulin syringes.[45]

Insulin pens and caps

The development of smart insulin pens such as Eli Lilly's Memoir or Luxura, Novo Nordisk's NovoPen6 or Echo, and Medtronic's InPen enables the time and insulin

dose of each injection to be recorded. The data can be linked to a smart device or to the cloud, and information can be transferred for viewing by physicians. Temperature alert and insulin expiry date are also useful functions found with some of these pens.

Insulin caps and attachments are variations that allow existing insulin pens to be transformed into smart pens. The Bigfoot Unity System, recently approved by the FDA, uses Smart Caps that are compatible with most types of short- and long-acting insulin pens, and tracks their usage. The cap doubles up as a continuous glucose monitor (CGM) scanner, alerting users to fluctuating glucose levels, and recommends insulin doses. Another notable smart cap undergoing clinical trial is the Go-Cap by Common Sensing that is attached to disposable insulin pens. The pen uses light in the cap to sense the amount of glucose present and records the dispensed insulin. Observational real-world studies on Swedish T1D subjects indicated that the smart pens may have improved the time in range (TIR) blood glucose level and increased medical concordance on treatment.[46]

CGMs

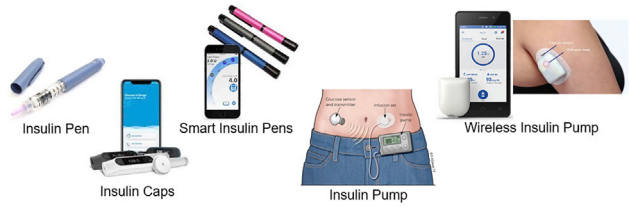
A CGM records glucose levels in the interstitial fluid continuously over a period of time. It consists of a sensor inserted subcutaneously and is attached to an external transmitter. The real-time information captured every 5 min is transmitted continuously or upon request to a receiving device or to the cloud. CGMs are fast becoming an integral part of diabetes management, especially in developed countries, with the aim of empowering the patient. This has improved patient compliance, motivation, and monitoring by caregivers and physicians, and reduced hyperglycemic or hypoglycemic episodes. The most common types of FDA-approved CGMs such as FreeStyle Libre, Freestyle Libre 2 (Abbott), Dexcom G6 (Dexcom), and Guardian Connect (Medtronics) record data for up to 14 days, most triggering alarms when the user is hypoglycemic. The Guardian Connect is linked with a third-party app, Sugar.IQ, that uses analytics to personalize the recommendations for every individual with diabetes. Sensionic's Eversense and Eversense XL sensors are implanted subcutaneously by practitioners and can measure glucose for 90 and 180 days, respectively. It is undergoing clinical trials to determine whether it can last 365 days. Among these CGMs, the Medtronic and Sensionic devices require calibration with a blood glucose monitor twice per day, at least every 12 h. A summary of the commonly available devices and their functions can be found in Table 2. Overall, the use of CGMs has resulted in better glycemic control[47] and reduced hypoglycemia episodes of adults on insulin therapy compared to standard blood glucose monitors.[48,49] The main obstacle currently is the high cost, data overload to the patients, and inconvenience of application and wearing of the devices.

Insulin pumps

Continuous insulin delivery systems, also known as continuous subcutaneous insulin infusion (CSII) pumps, have been widely tried and tested in North America and Europe. These pumps are used with CGMs to deliver short-acting insulin at a basal level to the body. The first-generation pumps deliver a fixed dose of insulin to the body, posing a risk of hypoglycemia. With the development of more sensitive sensors and algorithms to predict the trend of low blood glucose and suspend insulin delivery when hypoglycemia is imminent, these pumps have gradually gained favor with and the confidence of their users. There are two categories of new-generation insulin pumps: the open loop pumps, also known as the sensor augmented pumps (SAPs) and the closed-loop pumps, frequently known as artificial pancreas. Until recently, the insulin pumps were somewhat cumbersome, as the insulin was housed with

Table 2. Summary of the commonly available insulin devices and their functions

	Abbott Freestyle	Abbott Freestyle 2	Dexcom C6	Eversense	Medtronic Guardian Connect
Duration of wearing sensor and/or transmitter	up to 14 days	up to 14 days	sensor: up to 10 days transmitter: up to 3 months	90 days (XL: 180 days)	up to 7 days
Application of sensor/transmitter	self; sensor and transmitter all in one; easy to apply; 1-time use	self; sensor and transmitter all in one; easy to apply; 1-time use	Self	minor procedure; inserted by healthcare physician	self; separate sensor (1-time use) and transmitter (rechargeable up to 1 year or 22×)
Warm-up time	1 h	1 h	2 h	24 h	2 h
Calibration	No	no	optional, but not required with sensor code	2×/day; at least every 12 h interval	2×/day; at least every 12 h interval
Receiver	reader or phone app; needs to scan and read at least every 8 h for data to be stored	reader or phone app; needs to scan and read at least every 8 h for data to be stored	phone app	phone app	phone app
Alarms on high and low	No	yes	Yes	yes; vibration on arm in addition to alarm on app	yes
Stand alone	yes	yes	Yes	yes	yes
Insulin pump integration	no	pending (Tandem's t:slim X2 insulin pump)	yes; Tandem's t:slim X2 insulin pump Omnipod DASH	no	no
Age, years	≥18	≥18	≥2	≥18	≥14
Data sharing	via mobile app but not with reader	via mobile app only	Yes	yes	yes
Drug interference	vitamin C, salicylic acid	vitamin C, salicylic acid	acetaminophen/paracetamol, hydroxyurea	tetracycline	acetaminophen/paracetamol



	Traditional Insulin Pen	Smart Cap	Smart Pen	Sensor Augmented Pump	Closed Loop Insulin Pump
Track insulin in device	x	√	√	√	√
Track BG and injected dose	x	√	√	√	√
Calculate doses to inject	x	√	√	√	√
Check BG reminder	x	√ or linked to CGM	√ or linked to CGM	Integrated with CGM	Integrated with CGM
Generate report	x	√	√	√	√
Temperature alert for insulin	x	√	√	√	√
Short acting insulin	√	√*	√*	√	√
Basal insulin	√	√*	√*	x	x
Cost	+	++	++/+++	++++	+++++

* Separate pens

Figure 2. A summary comparing the various insulin delivery devices
CGM, continuous glucose monitor; BG, blood glucose.

the pump and the subcutaneous delivery was via tubing. The new version of patch pump such as Omnipod DASH is tubeless and wireless, with the insulin housed in a disposable reservoir sitting directly on the skin, and delivered via a cannula that is out of sight.[50]

The SAPs consist of an insulin pump, a CGM, and a transmitter. While the basal short-acting insulin is being pumped continuously, the patient would still need to inject a bolus of short-acting insulin before each meal. The hybrid closed-loop system has a suspend before low (blood glucose) function in systems such as Medtronic Minimed 670G and 770G with Smart Guard that stops insulin delivery when the glucose value is predicted to reach $\geq$1 preset hypoglycemic values within 30 min, and resumes when the sensor glucose reading recovers within a 2-h period. These hybrid closed-loop systems still require the patient to calculate and inject boluses of insulin pre-meal. Clinical trials reported a reduction in hypoglycemic episodes over a wide age range of T1D subjects who had suboptimally controlled glycemia or who were prone to hypoglycemia when compared to a control group using pumps without a real-time CGM.[51,52] The Control-IQ closed-loop system from Tandem Diabetes Care uses algorithms that predict and automatically administer correction boluses when glucose is too high, and the "Sleep Activity" predictive mode to deliver basal insulin throughout the night to achieve near-normal glycemia each morning. The incorporation of Control-IQ to the hybrid closed-loop systems (e.g., Medtronic 780G with Tandem Control-IQ) eliminates the patient requirement to calculate and inject boluses of insulin. In comparison to the use of SAP with CGM, TIR is significantly higher in T1D patients using closed-loop systems.[53] A summary showing the different types of devices for insulin injection and infusion is found in Figure 2. The major hurdles for the use of such devices are their affordability and the need for comprehensive patient education and training in using such systems. Malfunction of the pumps is also an issue, which requires patients to have alternative emergency plans in times of malfunction(s).

A bi-hormonal artificial pancreas controls the infusion of insulin and glucagon via two pumps with calculations from CGM and a control algorithm. In a small crossover study with 10 subjects, the bi-hormonal pump resulted in better glucose control and longer TIR during the nighttime as compared to the standard insulin pump.[54] The bi-hormonal artificial pancreas is a "true" closed-loop system for which there is no need for an external bolus even post-prandial and/or post-exercise.[55] The system requires refinement as there are four insertion points for two sensors, insulin, and glucagon pumps. The timely development and approval of room temperature-stable glucagon GVOKE (Xeris Pharmaceuticals) may contribute to the stability of glucagon in the closed-loop system. Longer-term studies comparing it to either SAP or the closed-loop insulin-only pump would shed light on its efficacy and applicability in real-life scenarios.

With the many types of approved advanced technologies in the treatment of diabetes and a flood of literature with findings and claims, the American Association of Clinical Endocrinology (AACE) set up a task force comprising medical experts "to provide evidence-based recommendations regarding the use of advanced technology in the management of persons with diabetes mellitus to clinicians, diabetes-care teams, health care professionals, and other stakeholders." [56] These guidelines will be updated once every 3 years to ensure that the clinicians and individuals using the technologies are up to date with the information on the use and interpretation of the data gathered.[56]

Apart from the injectable and infusible insulin, there have been other developments exploring different routes of administering insulin in a less invasive manner. They are used as a complementary approach to the existing injection or infusion, reducing inconvenience, pain, and risk of resistance or infection resulting from multiple daily injections (MDIs).

Inhaled insulin

The first inhaled insulin, Exubera, in the form of inhalation powder was approved by the FDA in 2006. It was a rapid-acting insulin and when used in conjunction with Ultralente, a long-acting insulin, showed similar efficacy when compared to the standard subcutaneous regular/NPH insulin. However, there was some deterioration in lung function in patients who took Exubera in the first 3 months, although their condition did not worsen for up to 2 years of monitoring. Subsequently, a higher incidence of cough was also noted in the Exubera group.[57] Adverse complications such as coughs, increased risk of infections, pharyngitis, rhinitis, and the possible risks of an increase in lung cancer were observed. It was withdrawn from the market in 2007 due to poor sales.

In 2014, another inhaled insulin (Afrezza, also known as Technosphere insulin) was approved by the FDA. Afrezza is an ultra-rapid-acting insulin formulation for prandial use. The insulin-loaded microparticles are inhaled directly into the alveoli, and takes effect within 30 min. It is then cleared by the body within 90 min. There has been renewed interest in studying and comparing the efficacy of Afrezza to injected insulin such as lispro. Results indicated that hypoglycemia is reduced in the group using Afrezza.[58,59] However, coughs seemed to be a persistent side effect of inhaled insulin. More long-term safety and efficacy studies would therefore be desirable.

Oral insulin

Oral insulin capsule is an attractive alternative for patients who are fearful of needle pricks. The major hurdles to overcome in the formulations of insulin are the instability

and limited absorption in the gastrointestinal tract. At the same time, they must be biocompatible and biodegradable. Capsulin oral antidiabetic drug (OAD) and IR use the Access delivery system that was reported to be more resistant to degradation by gut proteases, thereby increasing the absorption of macromolecules, peptides, and proteins across the intestinal wall and be transported to the liver via the portal system. These enteric-coated capsules are in Phase IIb and II clinical trials, respectively, for T2D. The ORMD-0801 (Oramed Pharmaceuticals) is also an oral capsule that prevents enzyme degradation and increases intestinal absorption. It is entering Phase III clinical trials to evaluate its safety and efficacy for the treatment of T2D (NCT04754334).

Another interesting development is a lipid nanoparticle-based oral hepatocyte directed vesicle (HDV) insulin (Diasome) that directs the encapsulated insulin to the liver. A Phase II/III clinical trial was completed in 2009 to assess the safety and efficacy of HDV oral combined with OAD, compared to placebo with OAD therapy. The results showed significantly reduced mean postprandial plasma glucose area under the curve (AUC) values compared to placebo, with no safety concerns. However, there was a lack of dose response with increasing insulin concentrations.[60] The company has since re-purposed the formulation to be used in injections and infusions, and achieved good outcomes in glycemic control for T1D patients in the Phase IIb clinical trial NCT02794155.[61]

Transdermal insulin administration

Transdermal administration is another approach that would help patients with needle phobia due to pain and discomfort caused by injections. We recommend readers to the two recent reviews describing the different strategies and formulations of transdermal insulin delivery.[62,63] Of these, several versions of jet injectors have completed the final phases in clinical trials, and products including Vitajet 3, InsuJet, Injex, and Comfort-In Needle Free Injectors are available on the market for individuals living with diabetes. Insulin is loaded into a cartridge via adaptors from insulin pens or vials and connected to spring-loaded devices. The push of a button releases a high-pressure jet stream of insulin into the subcutaneous region of the skin. Serum glucose levels with use of jet injectors were found to be consistently lower than those using syringes in insulin-dependent individuals with diabetes.[64–66]

Insulin gene therapy

Beyond daily exogenous IR, the search is on for a more durable "cure." This leads us to newer modalities such as insulin gene- or cell-replacement therapies, or transdifferentiation using pancreatic transcription factors to reprogram cells either *ex vivo* or *in vivo* to produce insulin. Successful *in vivo* transdifferentiations performed in diabetic rodents include liver to β cells,[67] acinar to β cells,[68] and α to β cells.[69] This section focuses on insulin gene therapy for T1D, which started in the 1990s and was continued by different groups of researchers over the past 25 years.[70] There are two approaches used: *in vivo* and *ex vivo* transfer of the insulin gene. Although refinement of the method has been made in various diabetic animal models, it has remained an experimental protocol. Nevertheless, it is a potentially valuable strategy offering one-time administration for long-term insulin expression and providing a stable endogenous source of insulin in individuals with diabetes.

In vivo *insulin gene therapy*

T1D is an autoimmune disease with the presence of autoantibodies, of which the islet cell antibodies (ICAs) have been found to react against the cytoplasmic proteins in the β cells. Therefore, insulin gene expression in extrapancreatic sites is the logical

step to begin with. The initial gene therapy experiments made use of constitutive promoters and expression in the liver. The aim was to provide basal insulin levels in the liver via gene transfer, which would significantly inhibit ketogenesis and restrain lipolysis, thereby having normalizing effects on blood glucose and fat metabolism.[71,72] The absence of β cell-specific proinsulin processing enzymes was solved by using a modified furin-cleavable insulin gene. Dong et al.[73] subsequently showed that insulin gene therapy at the basal level could be used as a strategy to improve the condition of diabetic rats treated with conventional insulin therapy. The use of strong constitutive promoters with a modified furin-cleavable insulin gene has also been shown by many groups to ameliorate blood glucose in diabetic animal studies.[74,75] However, modified liver cells lack secretory vesicles and cannot rapidly secrete insulin granules in response to glucose. Thus, the absence of dose regulation and feedback inhibition remain important hurdles.[76]

Subsequent reports were more ambitious in trying to mimic the β cells further by using glucose-responsive promoters.[77,78] The immediate glucose-responsiveness upon glucose challenge observed in β cells has not been recapitulated by the cells modified to be glucose responsive *in vitro*. The glucose-responsive promoter activity gradually increased and only peaked at 10 h post-glucose challenge.[79] The glucose responsiveness observed *in vivo* has been attributed by many groups to the use of glucose-responsive promoters. However, we and others have shown that a similar glucose tolerance profile was also observed in mice treated with insulin under a constitutive promoter.[79,80] Furthermore, hypoglycemia remained a risk even with glucose-responsive promoters.[79,81] The main shortcoming of present-day insulin gene therapy strategies remains the absence of insulin secretory granules, unlike that in β cells. There is no biphasic burst release of insulin even when the insulin gene is under the control of a glucose-responsive promoter.

Another challenge of using gene therapy for diabetes lies in achieving a perfect or optimal dose with a single administration. Plasmid[82] and mini-chromosomes[83] are non-viral delivery strategies that have been explored but are either invasive or lack translatability to the clinics. Many viral vectors, when coupled with tissue-specific promoters, can deliver insulin very efficiently into target sites such as the liver,[84] skeletal muscle,[85] and pancreas.[86] Lentiviral vector integrates into the genome and expresses the transgene stably.[74,86–88] Non-integrative adenovirus can last for at most a few months.[71,77,89–93] While adeno-associated virus (AAV) and AAV hybrid vectors are more long-lasting,[76,80,81] they prevent re-administration as a top-up strategy due to antibodies formed against the viral vectors. Repeated dosing strategies such as using a different AAV capsid,[94] neutralization of antibodies,[95] or immune suppression[96] are being studied.

We are of the opinion that the safer approach for hepatic insulin gene therapy, even with glucose-responsive promoters, would be to use them as a source of basal insulin coupled with pre-prandial insulin injections or one of the less invasive approaches such as inhalation of ultra-rapid-acting insulin. A safety switch that could turn off insulin temporarily during circumstances, such as fasting or surgery, would be necessary in treating humans.[97]

An interesting strategy was used by Bosch and team members,[85,98] who delivered AAV encoding insulin and glucokinase genes to the skeletal muscles of diabetic animals. In the presence of insulin, glucokinase acts as a glucose sensor, resulting in the transport and storage of glucose in the muscles when blood sugar is high. Streptozotocin (STZ)-induced diabetic dogs have now been treated for 8 years,

demonstrating good glucose control and absence of hypoglycemia. The product KT-A112 is still undergoing studies before commercialization.

Ex vivo *insulin gene therapy*

The early experiments of insulin cell therapy were carried out using non-endocrine cancer cell lines.[99,100] Although the proof-of-concept experiments showed that the insulin-producing cells could lower the blood glucose levels in animal models, they are not translatable to clinical trials due to the risks of tumor formation or uncontrolled proliferation of these cells *in vivo*. Subsequent experiments using primary cells such as fibroblasts,[101,102] intestinal K cells,[103,104] mesenchymal stromal cells,[105] and cord lining epithelial stem cells[106] ameliorated high blood glucose in diabetic mice. Fussenegger and colleagues have been working on synthetic switches and circuits incorporated into designer insulin-producing cells that are capable of turning on genes on-demand. The switches range from the food additive vanillin,[107,108] to light,[109] to electrical impulses,[110] to cool temperature (15°C–18°C) or cool sensation (menthol).[111] The site of transplantation for optimal insulin secretion, the timing and extent of insulin release, and the risk of rejection after engraftment remain important areas for consideration.

Insulin-secreting cell-replacement therapy

Donor islet transplantation

Cell-replacement therapy is thought to be a durable "cure." Cadaveric islet transplantation is one of the treatment options explored for diabetes patients.[112] The collagenase-based islet isolation procedure was pioneered by Lacy and Kostianovsky in 1967 using rodent pancreas[113] and later optimized to achieve mass isolation from larger mammals.[114] The islet isolation procedure had successfully derived islets for autologous transplantation into the portal vein of diabetic rats and non-human primates.[115,116] In the late 1980s, the first human islet transplantation was performed, resulting in brief insulin independence.[117] In 2000, the Edmonton protocol was established and has since become the mainstay in human islet transplantation.

To reduce the risk of rejection, xenoprotein-free reagents are used for the islet isolation and purification procedure. Freshly isolated islets pooled from two to three cadaveric donors are transplanted immediately to eliminate the need for islet culture.[118] The use of glucocorticoid-based immunosuppressants that often leads to diabetogenic effects is omitted from the Edmonton protocol and is replaced with an immunosuppressive regimen (sirolimus, low-dose tacrolimus, and daclizumab) that prevents the activation of endogenous T cells and the expansion of lymphocytes, to tackle both the autoimmunity against β cells as well as the rejection of allogenic grafts.[118] The 1-year follow-up with 7 T1D subjects who had received islets via the Edmonton protocol demonstrated insulin independence.[118]

Subsequently, an international multicenter trial (6 centers in North America and 3 in Europe) of 36 T1D patients using the Edmonton protocol was conducted (NCT00014911). Although 21 of 36 subjects attained insulin independence 1 year after transplantation, only 5 remained insulin independent 2 years later.[119] The Collaborative Islet Transplant Registry (CITR) has documented 677 islet transplantation events between 1999 and 2007. Among these, there was a trend toward improved primary efficacy and safety outcomes in recipients who received transplants in 2007–2010 compared with those in 1999–2006.[120] Another Phase III trial on islet transplantation reported that of 48 T1D patients who received islet transplantation, 11 achieved insulin independence by day 75, 25 by 1 year, and 20 at 2 years (NCT00434811).[121] A more recent Phase III trial was conducted in 24 T1D patients

who had kidney transplantation before receiving islet transplantation and an immunosuppressant regimen.[122] Among the 24 subjects, 5 rejected the transplanted islets within 1 year, while 15 achieved the primary endpoint of the study ($HbA_{1c} \leq 6.5$) and were free from severe hypoglycemic events.[122]

To date, islet transplantation has led to improved clinical outcomes in managing diabetes, averting the risks of hypoglycemia that accompany insulin therapy. It is observed that insulin independence is associated with a greater number of islet infusion and islet mass.[122] However, sustained insulin independence is not guaranteed.[119,121,122] Several reasons could explain the less-than-desirable outcome. First, early graft loss could be attributed to delayed oxygenation at the site of engraftment and an instant blood-mediated inflammatory reaction,[123] often resulting in the requirement for more than one islet infusion. Second, recurrent autoimmunity could play a role in the eventual graft loss. Last but not least, prolonged exposure to immunosuppressants could lead to impaired islet function.[119] While most islet recipients eventually return to insulin therapy due to varying degrees of graft failure, it is noteworthy that the insulin dose they require is substantially lower than before transplant, reducing the occurrence of the hypoglycemia events that accompany insulin therapy.

Notably, one T1D patient was reported to have achieved long-term insulin independence (>11 years) after receiving an islet transplant pooled from 2 donors. Yearly follow-up assessments revealed normal metabolic control since the patient was taken off insulin 3 months post-transplant.[124] This case study validated the possibility of achieving long-term insulin independence with allogeneic islet transplantation, which could be a potential cure for diabetes. However, scarcity of donors, risks of graft loss through immune response and/or delayed vascularization, and adverse effects from prolonged exposure to immunosuppressants remain key challenges to be overcome.

Islet xeno-transplantation

To overcome the scarcity of cadaveric human donors, xeno-transplantation of neonatal and adult pig islets has been explored. One neonatal pig pancreas yields ~50,000 islet aggregates,[125] and an estimated 4 donors will be required to treat a diabetic primate weighing 4–6 kg.[126] However, up to 800,000 islet equivalents can be purified from 1 adult pig,[127] making it possible to achieve single pig donor clinical xenogeneic transplant.[126] One of the major downsides of islet xeno-transplant is the potential risks of zoonosis. Porcine endogenous retroviruses (PERVs) are found to be integrated into the pig genome and could be challenging to fully eliminate.[128] Through the application of CRISPR-Cas9 editing, Yang et al.[129] reported genome-wide inactivation of 62 PERV genes in a porcine cell line and successfully downregulated transmission of PERVs to human cells by >1,000-fold. Using the same technique, PERV-inactivated pigs are generated via somatic cell nuclear transfer.[130] Besides addressing the safety concerns in xeno-transplantation, challenges similar to that of human islets are also present.

Human pluripotent stem cell (hPSC)-derived islet-like cells for transplantation

Islets isolated from multiple pancreases are usually required for a single transplant in humans. Hence, a reliance on human leukocyte antigen (HLA)-matched cadaveric islet sources is not likely to fulfill global clinical demands for insulin-requiring diabetes. In the past decade, there have been significant advances in deriving unlimited quantities of pancreatic islet/β-like cells from hPSCs, such as human embryonic stem cells (hESCs) or human induced pluripotent stem cells (hiPSCs).[131] However, despite

Table 3. Advantages and disadvantages of different islet/β-cell sources for cell-based treatment in insulin-dependent diabetes

Strategy	Advantage(s)	Disadvantage(s)
Cadaveric islets	supply of insulin from bona fide cells, no risk of hypoglycemia	scarcity of donors
	no need for routine insulin injections	require islets from 2–3 HLA-matched donors
		variable clinical outcomes due to different islet batches
		insulin independence is not guaranteed
		graft immune rejection, gradual loss of islet function
		require immunosuppressants
		side effects from immunosuppressants
		invasive, often require >1 infusion
Porcine islets	existing effective isolation method available to obtain good amount of islet equivalent from adult pig pancreas	risk of zoonosis
	potentially unlimited supply	special facility to house the animals, potential space constraints
		functionality in human requires further validation
		graft immune rejection expected
		require immunosuppressants
		ethical and cultural concerns
		invasive
hPSC-derived islet-like cells	unlimited supply of insulin-secreting cells	cells may not always be fully functional
	endogenous supply of insulin	residual undifferentiated cells will result in tumorigenicity
	amenable to gene-editing to create immune-evasive cells, potential in eliminating the need for immunosuppressants	cell dosage to be transplanted for full functionality remains to be determined
		limited clinical trials to confirm efficacy in humans
		potential allogeneic-mediated immune rejection
		invasive

sharing phenotypic and functional resemblance to bona fide human islets, these hPSC-derived islet-like cells still have inadequacies that prevent their immediate clinical use. Existing challenges, such as tumorigenic potential, functional immaturity, and achieving full compliance with current good manufacturing practice (cGMP) regulations enforced by the regulators will need to be overcome.[132] The functionally less-mature hPSC-derived β-like cells would also mean that a higher islet mass and/or more infusions will be required for a single transplantation. Nevertheless, these functionally less-mature β-like cells have been shown to restore normoglycemia in diabetic models. We recommend readers to recent reviews that have extensively discussed the various obstacles that stand between hPSCs and their clinical applications.[133,134] The pros and cons of various islet/β cell sources for transplantation are summarized in Table 3.

One key advantage of using autologous hiPSC-derived islet/β-like cells is the potential evasion of immune rejection. However, some considerations with this approach include the following: (1) the genetic background of hiPSCs should not contain diabetes-associated gene variant(s) that can cause inferior β cell function, (2) adult somatic cells used to generate hiPSCs may have accumulated unknown mutations throughout life that can affect the quality of differentiated β cells, and (3) every different hiPSC line may require customized optimization for differentiation and regulatory approval for GMP production.[132,135,136] Alternatively, should the approach

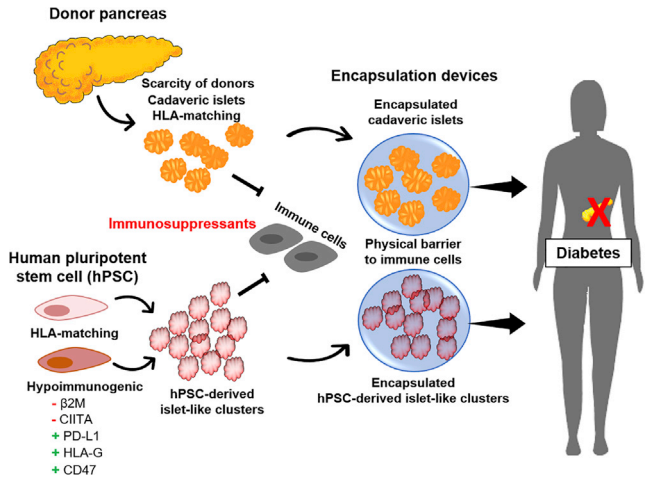


Figure 3. Strategies of cell-replacement therapy to treat diabetes

of using allogeneic hiPSC-derived β cell transplantation be adopted, then there is a need to devise strategies to avoid autoimmune rejections in T1D.

Strategies to evade host immune attack for cell-replacement therapy

When considering immune compatibility for cell transplantation, one can take appropriate steps at either the cellular level or the external environment. At the cellular level, the use of HLA-matched cells for allogeneic transplantation can greatly reduce the risk of immune rejection. There are three groups of HLA (HLA-A, HLA-B, and HLA-DR) and they are expressed in codominance to derive highly polymorphic HLA haplotypes in human. The Center for iPSC Research and Application (CiRA) in Japan has hiPSCs from ~50 carefully selected HLA haplotypes that are able to provide HLA-matched cells for >90% of the Japanese population. Similar efforts to create hiPSC biobanks are ongoing in Asia, Europe, and North America.[137,138]

Alternatively, hypoimmunogenic hiPSCs can be generated so that the differentiated β cells are less likely to or would not trigger an immune attack. As HLA molecules play important roles in mediating immune responses, manipulation of the genes encoding the accessory chains of the HLA molecules has been explored. The β-2-microglobulin (β2M) serves as the light chain of the HLA class I molecule. Deletion of *β2M* will thus lead to the deficiency of HLA class I molecule on the cell surface of hiPSCs and their derivatives.[139] While the *β2M* null hiPSCs will be protected from cytotoxic T cells, the absence of HLA class I molecules will cause the cells to be targeted by natural killer (NK) cells when these engineered hiPSCs fail to be recognized as "self."[139] To overcome this, Han et al.[140] used a strategy to selectively delete the highly polymorphic HLA class Ia and class II molecules (master regulator gene, *CIITA*), and introduced the immunoregulatory factors PD-L1, HLA-G, and CD47 to prevent the activation of T cell- and NK cell-mediated immune attack and engulfment by macrophages. Figure 3 summarizes the various strategies of cell-replacement therapy for diabetes treatment.

In T1D, it is still possible for autoreactive T cells to destroy HLA-matched or hypoimmunogenic hiPSC-derived β cells. Therefore, an important complementary strategy to protect transplanted cells is to create a physical barrier via an encapsulation material. This concept of cell encapsulation involves the use of selectively permeable membranes that allow for gaseous exchange, diffusion of glucose, insulin, and

nutrients to ensure graft survival while keeping the transplanted cells safe from the immune cells. The TheraCyte device was designed with an inner immunoprotective membrane to protect transplanted cells from allogeneic-mediated response. It also consists of an outer membrane that facilitates neovascularization.[141] Histological examination after 6 months revealed significant fibrosis within the device, which could pose a threat to long-term functionality of graft.[141] Encaptra is another device modified from TheraCyte by ViaCyte to enhance biocompatibility in the subcutaneous space to protect implanted cells against allo-immunity and auto-immunity. In 2014, a Phase I/II combination trial of hESC-derived pancreatic endocrine progenitors (derived from Cyt49 cells; PEC-01) encapsulated within PEC-Encap was initiated (VC-01, NCT02239354). Unfortunately, the grafts were found to have poor viability, likely attributable to hypoxia. A second trial, VC-02 (NCT03163511), involving systemic immunosuppressants was launched in 2017, in which PEC-01 was enclosed in PEC-direct comprising a perforated membrane that encourages the infiltration of blood vessels. Preliminary results reported that PEC-01 cells in a subset of recipients (6 of 18) were able to survive, engraft, and produce measurable C-peptide levels in T1D patients.[142] Recently, the preliminary data from one patient from VC-02 was presented at the American Diabetes Association's 81st Scientific Session, demonstrating a clinically relevant increase in glucose-responsive C-peptide levels.[143] Apart from the compelling preliminary outcomes with the clinical trials, ViaCyte announced a clinical phase agreement with Gore (a materials science company) in 2020 to develop a modified version of PEC-Encap to facilitate vascularization while eliminating the need for immunosuppression (https://viacyte.com/news-events/). Vertex is another company that recently announced their intention to perform clinical trials using functional stem cell-derived islet-like organoids (SC-islets) and a proprietary macroencapsulation device.[135]

Besides encapsulation devices, the microencapsulation of islets is being explored. Microencapsulation is thought to be advantageous in offering better oxygen and nutrient supply attributed to its higher surface area:volume ratio.[144] The implantation of alginate-microencapsulated neonatal pig pancreatic cell clusters (NPCCs) in a T1D patient has been reported to achieve long-term survival with a duration of >9.5 years.[145] To overcome the challenges in enclosing islets of varying sizes, conformal coating was explored. Tomei and colleagues reported that conformal-coated reaggregated SC-islets can reverse diabetes and maintain euglycemia in mice.[146] Similarly, Sigilon Therapeutics reported chemical modifications on the surface of microspheres that can prevent the initiation of immune response and pericapsular fibrotic outgrowth without the use of immunosuppressants, achieving a median islet viability of 90% (6 of 7) when the grafts were retrieved 4 months post-transplant.[147] Sigilon Therapeutics further explored a retrievable implant coated with a synthetic polymer that prevents fibrosis, demonstrating that transplanted rat islets within the encapsulation device are able to reverse diabetes and maintain euglycemia in immunocompetent mice for >75 days.[148] However, the long-term stability of hydrogel-based encapsulation remains to be studied.

In addition, to circumvent the risks of early graft loss from hypoxia, Beta-O2 developed Beta-Air, a bioartificial pancreas containing alginate-macroencapsulated human pancreatic islets that receive periodic oxygen supply through a smart pump connected to an implanted gas chamber.[149] Beta-Air is being tested in T1D patients and the clinical trial (NCT02064309) is projected to be completed in 2022. A second-generation device adapted for hPSC-derived islets is ongoing. Cell Pouch (Sernova) is a device that counters hypoxia differently. Designed as a scaffold formed into small cylindrical chambers containing removable plugs, Cell

Table 4. Strategies to evade host immune attack for cell-based therapy

Strategy	Approach	Advantage(s)	Disadvantage(s)
HLA-matched cells	HLA-matched cadaveric islets are selected for transplant (Edmonton protocol)	reduced risk of graft rejection	limited cadaveric islets available
			expected variation in clinical outcomes as a consequence of batch-to-batch differences
		excellent islet functionality	immunosuppressants may be required
	collection of a variety of HLA haplotypes for hPSC generation	a spectrum of carefully selected HLA haplotypes will be sufficient to cover a large population	establishment of biobanks will be required to house hPSCs of a huge spectrum of HLA haplotypes
		HLA-matched hPSCs readily available to derive pancreatic β cells for transplant	hPSCs of rare HLA haplotypes may not be readily available
			higher differentiated β cell variations expected as a consequence of line-to-line differences
			immunosuppressants may be required
			functionally less mature
			tumorigenic potential
Autologous islet cells	autologous-derived hPSCs for β cell differentiation	minimal risk of graft rejection	autoimmunity against β cell may be persistent
		no need for immunosuppressants	laborious, slow, and expensive to derive autologous-hPSCs for β cell differentiation
			functionally less mature
			tumorigenic potential
Hypoimmunogenic cells	deletion of *β2M* gene in hPSCs[139]	reduces activation of cytotoxic T cell immune response; prevents immune rejection	activation of NK cell killing
			functionally less mature
			tumorigenic potential
	deletion of HLA class I molecule, CIITA, and expression of PD-L1, HLA-G, and CD47 in hPSCs[140]	blunted cytotoxic T cell immune response; prevents immune rejection	complex genetic manipulation involved, hPSCs must be screened to ascertain absence of off-target effect
		reduces NK cell killing and macrophage engulfment	functionally less mature
			tumorigenic potential
Encapsulation	encapsulation device	encapsulation acts as a barrier to protect transplanted cells from immune attack	pre-vascularization required to prevent early graft loss
	macroencapsulation	acts as a barrier to protect host from tumorigenic cells	
	microencapsulation	adaptable to donor islets and hPSC-derived islet-like cells	
		potentially no need for immunosuppressant	

Pouch is pre-implanted subcutaneously to allow for incorporation with the host's tissue and microvessels. After removal of the plugs, the void spaces will be replaced with islets, providing a pre-vascularized environment believed to enable long-term survival and function of implanted islets.[150] Cell Pouch is undergoing clinical trial (NCT03513939).

Additional bioengineering strategies that complement and enhance insulin-secreting cell replacement therapies are being explored. However, these are beyond the scope of this review, which is focused on insulin therapy. We refer readers to our recent review, which elaborated on tissue engineering strategies for regenerative medicine in diabetes.[151] Table 4 summarizes the pros and cons of the various strategies in preventing host immune attack. The pros and cons of encapsulation strategy in promoting long-term survival of grafts (in the context of evading host immune attack) are also summarized.

Current clinical perspective: Will IR remain the last line of defense for diabetes?

The quest to develop better insulin therapies remains even after it was first discovered a century ago. There is an ongoing effort to develop new insulin formulations/analogs such as glucose-responsive insulin, and even novel routes of delivery. The end goal remains—to find a "near-perfect" glycemic control strategy that can be delivered in a safe and convenient manner. With the advent of CGM devices, faster- or longer-acting insulin with predictable pharmacokinetics coupled with automated delivery systems has been encouraging in delivering better clinical outcomes at greater convenience to patients. These advanced systems using artificial intelligence to predict and recommend the doses required will continue to improve and, in time, achieve a near-euglycemic algorithm for the individual. Having said that, the use of CGM devices will still require close monitoring by physicians, patients, and caretakers.

β cell failure remains the root cause of diabetes. The advent of hiPSCs coupled with improved differentiation protocols have facilitated the manufacturing of an unlimited quantity of pancreatic islets for cell-replacement therapy. However, it is not without challenges. These challenges include enhancing the long-term survival of grafts, as well as eliminating the need for long-term administration of immunosuppressants. Combined with the various encapsulation devices that are undergoing clinical trials, a multidisciplinary approach can provide a cure for diabetes through the delivery of pancreatic islets into insulin-dependent patients with the hope of achieving long-term glucose regulation. Gene therapy is another potential cure for diabetes. Since 2019, the FDA has approved several gene therapy products (https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products). With these initial successes, it is expected that gene therapies will become increasingly available as a treatment option for various diseases, including diabetes. While it may not offer a complete cure, insulin gene therapy could be a strategy to reduce or abolish the need for multiple and confusing insulin injection regimens. However, the safety and reliability of this approach have yet to be extensively investigated for clinical application. Nonetheless, it is envisioned that *in vivo* gene therapies offering a one-time fix for inherited gene defects is an attractive option for diabetes treatment.

While insulin replacement offers only a temporary solution, its effectiveness in diabetes management (not without side effects) is evident through the last century and hence is likely to remain the mainstay. The insulin gene- and cell-replacement strategies that can guarantee a cure are comparably more invasive and remain a work in progress. With more successful clinical trials ascertaining the safety of cell-replacement therapy and demonstrating that the elimination of daily insulin delivery can be achieved, we believe that this strategy may eventually be adopted as one of the therapeutic alternatives during the next century.

The advancement of new strategies to tackle insulin-dependent diabetes has delivered optimistic outcomes. However, a large proportion of individuals with diabetes are living below the poverty level, and many are unable or barely able to afford the currently available forms of insulin. The affordability of therapeutic options remains a barrier for these people to achieve improved health. We believe that there will not be a one-size-fits-all treatment in the next 100 years, but rather a diversified range of developments to cater to the disparate needs of individuals with diabetes. As we chart the next century for a viable insulin replacement, it is crucial to ensure that the upcoming strategies are safe, affordable, long-lasting, and effective. In

this way, we can liberate individuals from the daily battles in the war against diabetes.

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DECLARATION OF INTERESTS

A.M.J.S. is a consultant for ViaCyte Inc and DiaGon Inc. A.K.K.T. is a co-founder of BetaLife. All of the other authors declare no competing interests.

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