Considerations and Challenges of Islet Transplantation and Future Therapies on the Horizon

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Abstract

Islet transplantation is a treatment for selected adults with Type 1 diabetes and severe hypoglycemia. Islets from two or more donor pancreases, a scarce resource, are usually required to impact on glycemic control but the treatment falls short of a cure. Islets are avascular when transplanted into the hypoxic liver environment and subjected to inflammatory insults, immune attack and toxicity from systemic immunosuppression. The Collaborative Islet Transplant Registry with outcome data on over 1000 islet transplant recipients has demonstrated that larger islet numbers transplanted and older age of recipient are associated with better outcomes. Induction with T cell depleting agents and the TNF-α inhibitor Etanercept and maintenance systemic immunosuppression with mTOR inhibitors in combination with calcineurin inhibitors also appear advantageous, but concerns remain over immunosuppressive toxicity. We discuss strategies and therapeutics which address specific challenges of islet transplantation, many of which are at the pre-clinical stage of development. On the horizon are adjuvant cell therapies with mesenchymal stromal cells and regulatory T cells that have been used in preclinical models and in humans in other contexts; such a strategy may enable reductions in immunosuppression in the early peri-transplant period when the islets are vulnerable to apoptosis. Human embryonic stem-cell derived islets are in early phase clinical trials and hold the promise of an inexhaustible supply of insulin producing cells; effective encapsulation of such cells or, silencing of the HLA complex would eliminate the need for immunosuppression, enabling this therapy to be used in all those with Type 1 diabetes.

Introduction

Diabetes affects over 422 million people worldwide, has an adult prevalence of 8.5% and is the fastest increasing chronic disease with substantial economic impact [1, 2]. Type 1 diabetes (T1D) is an autoimmune condition characterised by the absence or near absence of circulating C-peptide and affects up to 15% of the diabetic population [3]. People with the condition are reliant on exogenous insulin therapy but complications of insulin treatment include severe hypoglycemia (SH) [4, 5]. SH, defined as a low blood glucose requiring external assistance associated with a blood glucose value < 70mg/dL (3.9mmol/L) [5, 6], occurs in 35-42% of T1D patients with a rate of between 90-130 episodes/100 patient years [7]. Repeated episodes of hypoglycemia leads to impairment of the counter-regulatory system with the potential for the development of hypoglycemia unawareness [8, 9]. Despite advances in technology which can have profound benefits for subgroups of patients particularly in the current era of continuous glucose monitoring systems and hybrid closed-loop systems, overall the prevalence of SH in people with T1D remains unchanged [10].

Islet transplantation, history, indications and outcomes

Allogeneic islet transplantation whereby islets are isolated from a donor pancreas and transplanted most commonly into a recipient with T1D, is a clinically proven intervention for T1D associated with recurrent SH. This can eliminate exogenous insulin injections, stabilise blood glucose control, prevent or diminish hypoglycemia, restore symptoms of hypoglycemia and halt the progression of T1D related complications [4, 11-14]. The history of islet transplantation and main clinical trials are outlined in **Figure 1** [13, 15-35]. Insulin independence is not a primary aim and 5 year insulin independence rates are < 50%, with attrition in islet function seen over time in the majority [36]. Nevertheless, minimal graft function protects against hypoglycemia [37-39]. Islet transplantation is associated with a reduction in the progression of microvascular complications, including neuropathy and

retinopathy [4, 40, 41]. Effects on nephropathy may be complicated by coexisting kidney transplantation and the impact of immunosuppression, the latter leading to an early decline in renal function; however, there is evidence to suggest renal outcomes stabilise in the long term [4]. Short-term studies have demonstrated a positive impact of islet cell transplantation on surrogate markers of macrovascular disease but this has not been examined in randomised controlled trials [4, 40]. This minimally invasive procedure has an excellent safety profile which may be considered in patients with co-morbidities that would not be fit enough to undergo the major intra-abdominal surgery involved in pancreas transplantation [42-44].

The first randomised controlled trial in islet transplantation demonstrated superior metabolic endpoints with improved hypoglycemic awareness versus insulin therapy and also improved quality-of-life [1, 34]. The requirement for immunosuppression is the main consideration and the increased risk of infections [45], nephrotoxicity [46] and the x4 fold risk of cancer [36], limits patient selection to those age ≥18<65 years without a history of cancer.

Challenges in islet transplantation

Multiple challenges exist in islet transplantation as demonstrated in **Figure 2** [47-67] and the procedure currently falls short of a cure for T1D. A donor pancreas contains approximately 1 million islets but following digestion, purification and culture of islets, <50% of this number are isolated [68-70].

Islets are avascular when transplanted into the liver and susceptible to apoptosis in the liver in the first few days peri-transplant [71, 72]. Following transplantation, islets are subject to oxidative stress, inflammation, including the instant blood mediated inflammatory reaction (IBMIR) and rejection from alloimmune and autoimmune mechanisms [70] and <60% of transplanted islets successfully engraft into the liver [73]. Angiogenesis commences at day 3 post-transplant and takes approximately four weeks to complete. Attrition in graft function is

seen post-transplant but is incompletely understood [32, 36, 70, 74-77]. The liver is a tolerogenic organ and one of the only sites where transplanted islets have been associated with insulin independence [78, 79]. Typically, islets from ≥2 donor pancreases given as sequential infusions are required to impact glycemic control however successful single graft islet transplantation is seen [36] and has been reported in a number of single centres [80, 81] as well as in the Collaborative Islet Transplant Registry (CITR) [36]. A recent study in islet transplant recipients receiving two versus one islet graft demonstrated that despite transplant recipients of two grafts receiving 1.9 times the number of islets compared to single graft recipients (median(IQR) 12,218(9,291-15,417) versus 6,442(5,156-7,639) IEQ/kg; $p<0.0001$), 90 minute C-peptide concentrations following a mixed meal tolerance test at 1 year post first transplant, were not significantly different [80]. Furthermore, the numbers of islets received in the first graft were associated with graft function in those receiving one and two grafts [80]. This result although requiring confirmation, highlights the importance of the first transplant and many programs aim to deliver high numbers of islets with the first islet infusion.

Islet transplant programs have pooled their data and the CITR has allowed meaningful interpretation of transplant outcomes. This registry (latest CITR – 2015, 10th annual report [36]) consists of outcome data from 1086 patients world-wide that have undergone islet allotransplantation. Donor and recipient selection meet strict criteria [48] as do release criteria of islets for transplant, which include sterility standards, numbers isolated (≥5,000 IEQ/kg), purity ≥30% and viability ≥70% [82]. Induction and immunosuppression regimens differ from centre to centre and over time, which has allowed an understanding of the impact of these factors on islet transplant outcomes.

Factors associated with islet transplant outcomes are discussed and therapies that may address challenges in islet transplantation are highlighted and shown in **Figure 2.**

Factors known to influence islet transplant outcomes

Islet numbers: Islet numbers ≥325,000 islet equivalent units (IEQs) and >10,000 IEQs per kilogram recipient body weight are associated with insulin independence [83] although results differ from centre to centre [84]. Islet mass at first transplant appears critical [14, 84] and some programs aim for greater first islet transplant mass [14]. A time interval of >6 months between the first and second transplant may negatively impact on transplant outcomes [84]; donor specific antibody mediated rejection may play a role but has not been conclusively shown [84].

Age of recipient: recipient age ≥35 years are associated with better outcomes likely related to diminished autoimmune attack of transplanted β-cells [85]. Studies have demonstrated a negative correlation between increasing age and islet cell autoantibody positive status in Type 1 diabetes, consistent with this observation [84]. The mean(SD) age of people receiving islet allografts in the CITR is 46(±10.5) years [36].

Continuation of insulin therapy post-transplantation: in the Edmonton protocol published in 2000, insulin therapy post-transplant was withheld unless serum glucose concentrations exceeded 11.1 mmol/L, at which stage another islet transplant was undertaken [13]. Currently, in order to in theory limit the stress on the transplanted islets, insulin therapy is now reinstated following islet transplant until satisfactory glucose control is observed [86]. However, controlled trials in humans in this area demonstrating the benefits on islet survival post transplantation are lacking but nevertheless the administration of insulin to control glucose concentrations is pragmatic and overall beneficial.

Induction with T-cell depletion and/or TNF-α inhibition: induction with the anti-CD52 antibody alemtuzumab that targets mature lymphocytes is associated with lymphopenia and a decrease in *de novo* antibody formation post allotransplant [75] and this, in combination with the TNF-α inhibitor Etanercept, is associated with positive long-term graft outcomes. Anti-thymocyte globulin (ATG), is also associated with improved graft function [55]. Anti-CD3

agents block T cell differentiation and proliferation, induce regulatory T cells [87] and have recently been shown to preserve C-peptide concentrations in those with newly diagnosed type 1 diabetes [88] and are currently being utilised in early clinical trials in islet transplantation.

Mammalian target of rapamycin (mTOR) inhibition in combination with calcineurin inhibitors (CNI): mTOR inhibitors such as sirolimus were used in the original Edmonton protocol and were thought to have decreased renal toxicity and diabetogenic effects [13, 89]. When combined with CNIs such as tacrolimus, an agent that impairs transcription of (IL)-2 and several other cytokines in T lymphocytes, mTOR inhibitors are associated with positive islet graft outcome measures. mTOR inhibitors are now less commonly used: previously 86.9 % in 1999 to 2002 to 59 % in 2011 to 2014 to no reported use in 2015-2018 [90]. This decrease is due to less well-tolerated side effects without the advantage of better outcomes [89]. The most common immunosuppression now is mycophenolate mofetil (MMF), an inhibitor of inosine-5'-monophosphate dehydrogenase leading to inhibition of proliferation of T and B lymphocytes, with suppression of cell-mediated immune responses and antibody formation in combination with CNIs. Adverse effects of tacrolimus include insulin resistance and renal dysfunction, which are ameliorated with dose reductions but β-cell mediated toxicity is a concern [4] and alternative immunosuppressive agents hold promise.

Therapies in early clinical trials and on the horizon

Human embryonic stem cell (hESC) derived islets are in early phase 1/2 clinical trials and this may lead to an inexhaustible supply of islets which could transform the field [91] although tumorigenicity, while unlikely, is a concern [92]. Encapsulation of hESC islets could eliminate the need for immunosuppression [93, 94], as could HLA silencing [66, 95-99], both of which would enable children to be treated. Transplantation of hESC islets in a device in the subcutaneous space is theoretically advantageous but in practice has been difficult due

to scar formation around the site limiting the release of insulin [100]. Alternative strategies [101] and exploitation of specific biomaterials in the subcutaneous site are a focus of research [60], as are human induced pluripotent stem cell (hIPSC) [99] and xenogeneic sources of islets [50, 60]. Bioscaffolds are becoming increasingly investigated as a potential aid to islet engraftment – for example, dexamethasone-loaded microplate enriched collagen coated polydimethylsiloxane scaffolds enhance islet function and prolong graft survival [102]. The manipulation of self-reactive T-cells to delete the responsiveness to self, known as tolerance, is also being investigated [103].

Most adjuvant therapies that may improve human islet transplant outcomes are at the preclinical phase of development. Cellular therapies including mesenchymal stem cells (MSCs) as well as their products [104] hold particular promise as they have already been used in humans for other conditions [105]. MSCs are pro-regenerative, anti-inflammatory and immunomodulatory [67] enabling in theory a reduction in the dose of immunosuppression [104]. Autologous bone marrow derived MSCs transplanted in people with new onset T1D where there are remaining β-cells with detectable C-peptide, shows that C-peptide concentrations are preserved to a greater degree than when MSCs are not given [106]. Meta-analyses of islet transplant outcomes in humans have shown that less pure islet preparations, where there are conceivably more pancreatic MSCs, are associated with better islet graft function [107]. Other cellular therapies including regulatory T cells may also hold promise as a co-therapy for islet transplantation due to their pro-regenerative, anti-inflammatory and immunomodulatory properties and have been given in man [108].

Modulation of the liver niche with growth factors [109] and anti-inflammatory agents [110] have been used with some success and polymer properties may be exploited to regulate release of such factors when islets are immediately transplanted and particularly vulnerable to apoptosis [109, 111], but these are still at a very early pre-clinical stage. Accelerating the vascularisation of transplanted islets with a number of approaches including gene therapy

methods [112] may be a relevant strategy to accelerate islet engraftment and may improve transplantation outcomes.

Conclusions

Islet transplantation stabilises glycemic control, reduces hypoglycemia and restores hypoglycemic awareness. However long term insulin independence rates are low. Despite its success, major factors limit the application of islet transplantation including scarcity of appropriate organ donors, poor islet engraftment rates, long-term deterioration in islet function, and formation of allo- and auto-antibodies in patients receiving multiple grafts. The use of immunosuppression is associated with an increased risk of cancer and infections and limits the procedure to selected adult patients. Since no replenishable source of islets or β-cells exists for routine clinical use the best use of donated pancreases is imperative; adjuvant cellular therapies have shown benefit in pre-clinical studies and these co-therapies are on the horizon. Other more experimental techniques targeting the liver niche hold promise. The field of islet transplantation may be transformed by the use of hESC islets, already in early stage clinical trials, which would enable more people to be treated to achieve insulin independence. The use of these islets as well as other alternative sources of islets with no requirement for immunosuppression would open up the possibility of islet transplantation for all with T1D including children.

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Figure 1. Timeline of important developments and clinical trials in islet transplantation.

The timeline of significant studies, developments and clinical trials in islet transplantation are shown.

Figure 2. The challenges and potential future therapies for islet transplantation.

The main challenges of islet transplantation are demonstrated along with potential future solutions. Such solutions include expanding the source of insulin producing cells to meet demand, reducing inflammation post islet transplantation using pharmacotherapies and cell therapies, using alternative immunosuppression and eliminating the need for immunosuppression by using biomaterials and HLA silencing.

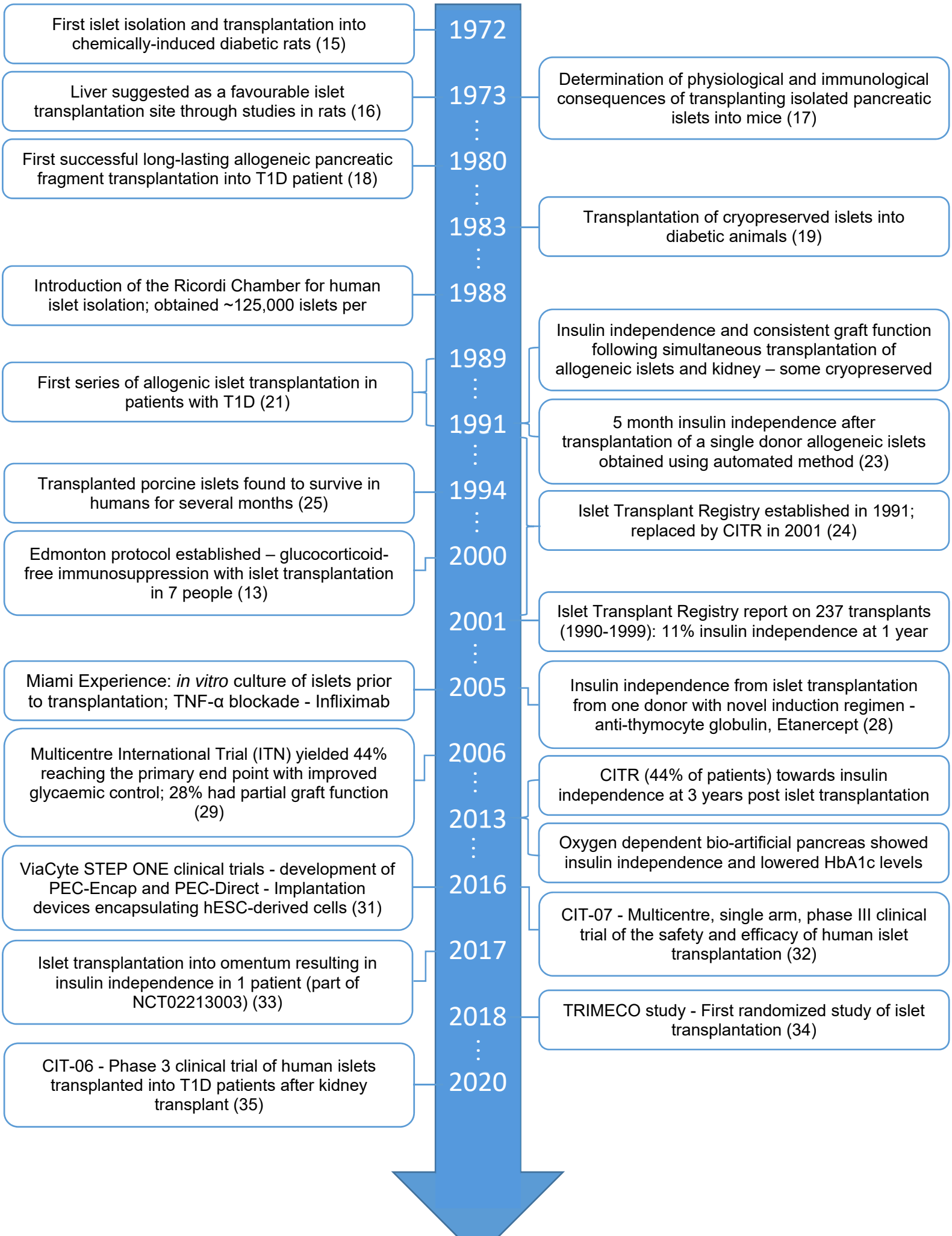

Figure 1: Timeline of important developments and clinical trials in islet transplantation.

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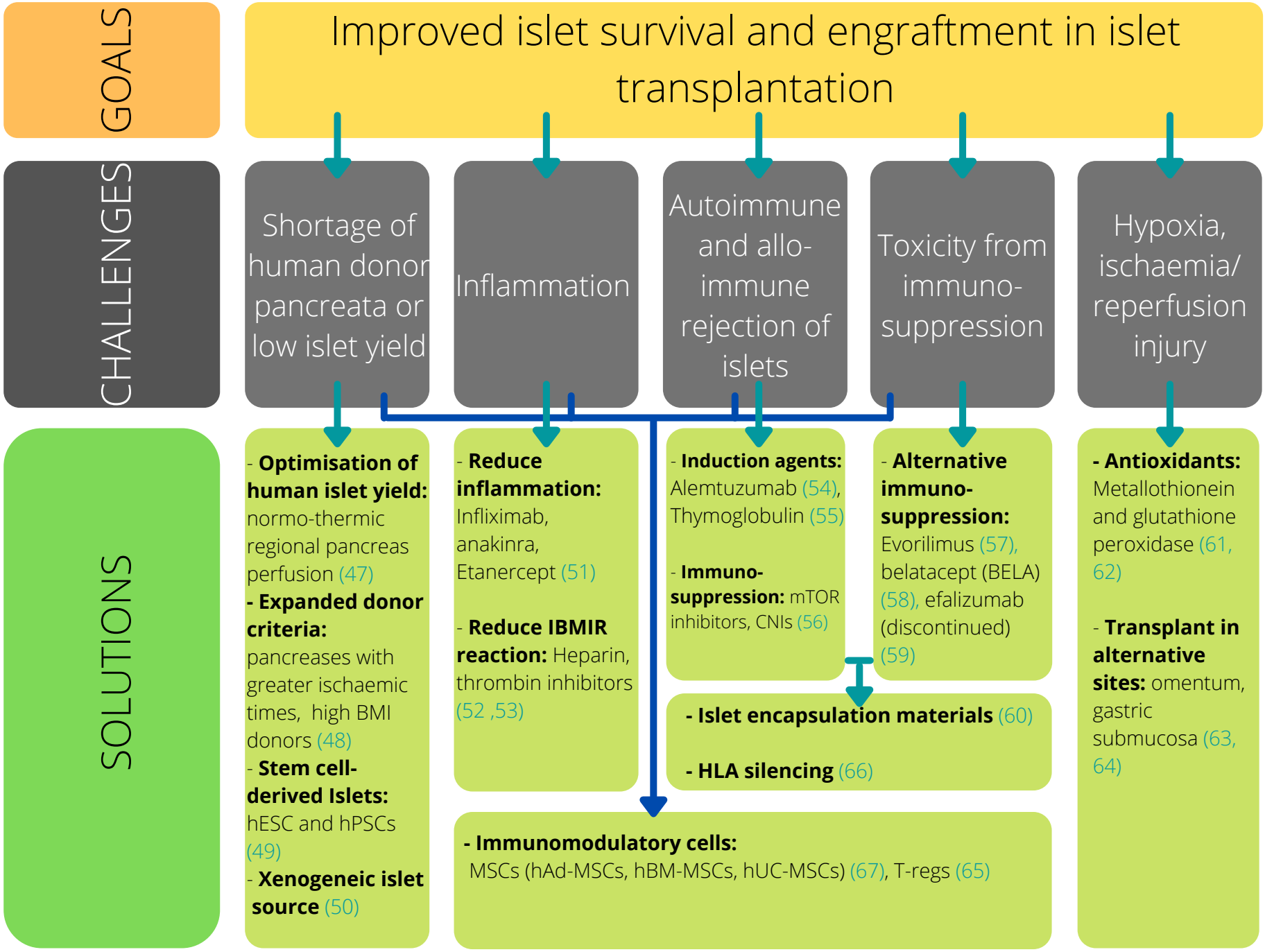
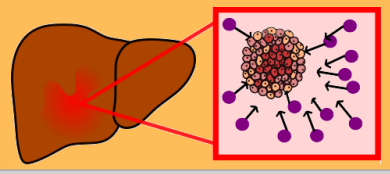


Challenges of islet transplantation and future strategies

Current problems

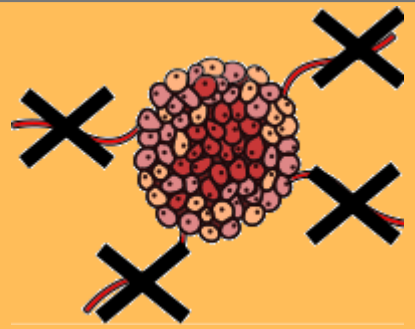
Inflammation



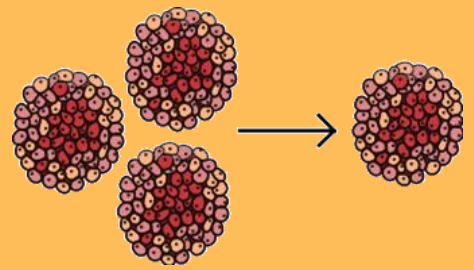
IBMIR (instant blood mediated inflammatory response), auto-/allo-immune response

Immunosuppression

Poor vascularisation

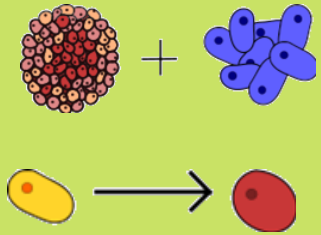


All contribute to **Islet loss**



Future directions

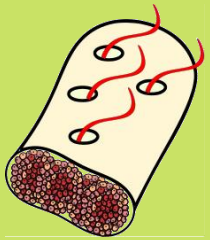
Cell therapies



MSCs; T-regs

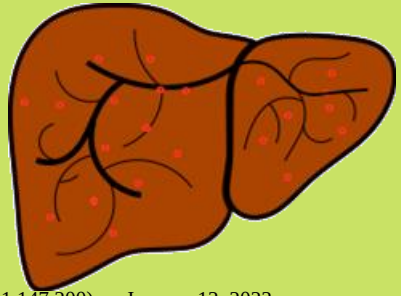
hESC islets

Biomaterials



Cell encapsulation

Liver niche modulation



Alternative immunosuppression