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Use of Culture to Reach Metabolically Adequate Beta Cell Dose by Combining Donor Islet Cell Isolates for Transplantation in Type 1 Diabetes Patients

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Author Roles: D.L., B.K. and P.G. contributed to the study design, literature research, data collection, data analysis and interpretation and wrote of the manuscript. R.H., U.V.d.V., and Z.L. researched data and reviewed the manuscript. D.J.-T.-T., G.M. contributed to the data research and review of the manuscript. B.L., C.D.B., L.C. contributed in patient recruitment, data research, and review of the manuscript. C.M. reviewed the manuscript. D.P. contributed to the discussion and edited the manuscript. P.G. and B.K. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Abbreviations

ATG	Antithymocyte globulin
BMI	Body mass index
GV	Glycemic variability
h	Hours
IEQ	Islet equivalents
IQR	Interquartile range
IS	Immune suppression
MMF	Mycophenolate mofetil
PT	Posttransplantation
TAC	Tacrolimus
T1DM	Type 1 diabetes mellitus

ABSTRACT

Background : Clinical islet transplantation is generally conducted within 72 hours after isolating sufficient beta cell mass. A preparation that does not meet the sufficient dose can be cultured until this is reached after combination with subsequent ones. This retrospective study examines whether metabolic outcome is influenced by culture duration.

Methods : Forty type 1 diabetes recipients of intraportal islet cell grafts under ATG induction and MMF-Tacrolimus maintenance immunosuppression were analysed. One subgroup (n=10) was transplanted with preparations cultured for ≥96hours; in the other subgroup (n=30) grafts contained similar beta cell numbers but included isolates that were cultured for a shorter duration. Both subgroups were compared by numbers with plasma C-peptide≥0.5ng/ml, low glycemic variability associated with C-peptide≥1.0ng/ml, and/or with insulin independence.

Results : The subgroup with all cells cultured ≥96hours exhibited longer C-peptide≥0.5ng/ml (103 versus 48 months, P=0.006), and more patients with low glycemic variability and C-peptide≥1.0ng/ml, at month 12(9/10 vs. 12/30; P=0.005) and 24(7/10 vs. 6/30; P=0.007). In addition, 9/10 became insulin-independent versus 15/30 (P=0.03). Grafts with all cells cultured ≥96 hours did not contain more beta cells but a higher endocrine purity (49% versus 36%, P=0.03). In multivariate analysis, longer culture duration and older recipient age were independently associated with longer graft function.

Conclusions : Human islet isolates with insufficient beta cell mass for implantation within 72 hours can be cultured for 96 hours and longer to combine multiple preparations in orde to reach the desired beta cell dose and therefore result in a better metabolic benefit.

Introduction:

Islet beta cell transplantation has improved considerably in recent years but the duration of graft function remains heterogeneous. Both donor and recipient factors were identified as important factors determining long term results.[1-3] Concerning graft characteristics, a minimum number of IEQ/kg (or beta cells per/kg) in the graft is considered to be the most determinant factor of successful outcome.[2-4] Most centers will proceed with transplantation only when the procurement of the donor pancreas and subsequent isolation procedure lead to a satisfactory islet yield after which islets from this single donor are infused immediately or after overnight culture.[5-9]

In most transplant centers, the duration of islet culture preparations are is limited to 24 to 72 hours (h),[2,5,7,9-13] because of the progressive reduction in the absolute number of beta cells during culture, and due to the well-established relationship between graft function and the number of islet equivalents that are infused intraportally.[14] In these centers, the islets are not transplanted if a predefined number of IEQ is not reached after isolation. In regions with less than optimal islet donors (those of more advanced age and with lower BMI and longer mean stay in intensive care), such inferior isolation outcomes are common. Another option is to keep the preparations under culture for a longer duration.

In our islet cell programme, we combine cell preparations derived from various islet yields so that at least 2 million beta cells per kilogram are transplanted.[4] This strategy requires a longer culture time of the cells resulting in the possible disadvantage of losing functional beta cells during the culture or after transplantation.[15] Prolonged culture, on the other hand, allows the facilitation of planning of the infusion procedure and thus reducing the stress of patients in the pretransplant period. It is also possible to reduce the cytokine release symptoms related to ATG administration at the time of islet infusion, since the first administration of ATG can

be done 24 to 48 hours before. Maintenance immune suppression target levels are reached most easily during the first days post transplantation.

In this retrospective study, we investigated if longer culture time (≥96h) of the first combined graft (defined as the graft at initiation of immune suppression) led to the longer transplant survival.

Materials and Methods

Graft Recipients

Data were collected from 40 islet beta cell graft recipients with type 1 diabetes mellitus (T1DM) and hypoglycemia unawareness, who were transplanted beween December 2001 and January 2011 (Figure 1). These recipients were consecutively transplanted based on their waiting time and body weight since we aimed at transplanting at least $2x10^6$ beta cells per kilogram of recipient body weight. No changes occurred in recipient selection during the study period. All recipients had type 1 diabetes for more than 5 years, were plasma C-peptide negative (<0.09 ng/ml), showed a large variation in blood glucose levels (glycemic variability>25%), HbA1c >7% and had 1 or more chronic diabetes complications. Before the transplantation, autoantibodies for ICA, GAD and IA2,[16] T-cell autoreactivity to GAD and IA2,[17] HLA class I and II antibodies and lymphocyte subsets[18,19] were determined as previously described.[4] All patients were CDC-HLA class I negative and crossmatch before transplantation was negative in all patients.

The Ethics Committees of the Belgian Diabetes Registry (BDR) and the participating university hospitals approved the protocol. This study was performed according to the Declaration of Helsinki and institution review board approval was obtained (institutional review board protocol BK/3 and BK/136; clinicaltrials.gov NCT00623610 and NCT00798785). A written informed consent was obtained from every patient. The local ethical committee approved the protocols (CME 2005/118 2010/193).

Preparation of islet beta cell grafts

Donor organs were procured after brain death as well as circulatory death through the Euro transplant Foundation (Leiden, The Netherlands) and processed at the Beta Cell Bank in Brussels as previously described.[20] No changes occurred in donor selection criteria during the study period.

Islet cell-enriched fractions were cultured as described previously.[21,22] In short, fractions were suspended in T175 culture flasks with 47 ml culture medium (approximate 1 million cells / ml) in CO2 incubator at 37°C. Culture medium was Ham's F10 containing 0.5% human albumin, 7.5 mM glucose, 2 mM glutamine and 2 mM nicotinamide. During 2000 to 2007, 2% human AB serum was added during the first 1 or 2 days culture. Preparations used for 9 recipients were cultured at room temperature for 24 hours prior to implantation.

During culture, the preparations were analysed for their beta cell number and purity.[4,20,22] Data were used to select preparations that, after combination, would constitute a graft with minimal $2x10^6$ beta cells per kilogram of recipient body weight (Figure 1).[4] The final cellular composition and beta cell mass of each graft was determined on samples that were taken after the combination of different preparations just before implantation.[4] This final result was therefore obtained after infusion. Total cell number was determined with DNA assay (6.5 pg per cell)[4] before year 2007 and with nuclei count assay after 2007. The cell composition was determined by electron microscopy and immunocytochemistry. Beta cell number was derived from a total cell count and the percentage of insulin-positive cells in the preparations.[4,22] This method achieves a direct and specific assessment of beta cell mass but takes more time than the classically used IEQ method. We did not simultaneously measured IEQ values as these are questionable in cultured islet cell preparations, because their dithizone staining for beta cells becomes less intense due to degranulation and loss of zinc, and thus it becomes more difficult to distinguish from nonspecific staining. Additionally,

changes in the intercellular adhesion and extracellular volume in the cell aggregates interfere also with the correct comparison with measurements from freshly isolated preparations.

The final multidonor grafts were composed from cell preparations with each different culture times ranging from 16 to 511 h for the first graft and 19 to 821 h for the second graft. Ten patient received first grafts containing cells that were all cultured for at least 96 h compared to thirty who received grafts containing cells cultured for less than 96 h (Table S1, SDC, http://links.lww.com/TP/B946). All second grafts except 1, contained cells cultured for less than 96 h.

Transplantation protocol and immune suppression

Patients received in total 4.7 x 10^6 beta cells/kg body weight (IQR 3.8-5.7), most frequently via 2 intraportal injections with a median interval of 2.7 months (IQR 2.3–3.1) (Figure 1). None of the patients received more than 2 implants. The grafts were infused into the portal vein over 5–6 minutes via a catheter inserted in the umbilical vein (n=18)[23] or through subcutaneous trans hepatic puncture under ultrasound guidance (n=22).[24] The IS regimen was the same during the study period and consisted of induction therapy with antithymocyte globulin (ATG-Fresenius, Fresenius, HemoCare, Redmond,WA) and maintenance therapy with mycophenolate mofetil (MMF, Cellcept, Roche, Basel, Switzerland) or mycophenolic acid (Myfortic, Novartis, Basel, Switzerland) and tacrolimus (Prograf(t))®, Astellas Pharma Europe, Staines, UK).[4] A single dose of steroids (500 mg methylprednisolone) was administered 2 hours before each infusion. Antiplatelet and anticoagulant therapy was started 1 day after infusion and consisted of acetylsalicylic acid 100 mg once daily in all patients and additional low–molecular weight heparin was administered at a preventive dosage (0.4ml Fraxiparin once daily). In 3 patients undergoing laparoscopic implantation, heparin was added to the transplant medium at 70 U/kg with continuous infusion of heparin containing saline (10U/ml) at 500 U/h (50ml/h) up to 24h posttransplantation (PT). Doppler

ultrasonography of the portal vein and liver was performed within 24h PT to rule out bleeding and thrombosis.

Measurement of implant function

Graft recipients were followed in our outpatient clinic weekly until PT week 6, and every 2 weeks between PT week 6 and 12 and monthly thereafter. All patients performed 4- to 7-point glycemia profiles of home blood glucose monitoring. Glycemic variability (GV) was defined as the coefficient of variation of prebreakfast glycemia measured from home blood glucose monitoring during a 2-month period. Plasma C-peptide, glycemia and HbA1c concentrations were measured in the central laboratory of the Belgian Diabetes Registry in Brussels.[4]

The main outcome parameter was the duration of islet graft function, defined as plasma C-peptide positivity ≥0.5 ng/ml. Secondary outcome parameters were duration of plasma C-peptide levels ≥1ng/ml associated with low GV (<25%)[25] and duration of insulin independence. The duration for each endpoint was calculated by subtracting the start day of the endpoint from the day when it no longer fulfilled the criteria after 2 consecutive measurements.

Statistical methods

All values are expressed as median and interquartile range (IQR) or mean ± standard deviation (standard error of mean) when indicated. Factors that are, based on previous publications[2,3,16,17,26-35] related to graft function, (ie, patient characteristics, graft characteristics, as well as immune parameters including pretransplant cellular autoreactivity, presence of pretransplant autoantibodies and HLA antibody status pretransplantation) were selected for analysis. For the comparison of the baseline patient characteristics, Pearson's Chi-squared test was used for categorical data.

To evaluate the impact of culture time ≥96 h on graft survival, results were compared between patients that received first grafts with all cells cultured ≥96 h with those receiving first grafts with cells cultured <96 h. To evaluate the impact of age on graft survival the total population was divided in groups based on age quartiles: quartile 1 (<39 years), quartile 2 and 3 (39-52 years) and quartile 4 (>52 years).

Differences between varying points in time during the follow-up were calculated using Fisher's exact test for categorical data and Mann-Whitney U test for continuous data. All analysis was performed using SPSS (version 25.0) and the graphics were computed by using Graph Pad Prism (version 5.0). Analysis of correlations was performed using the Pearson's rank correlation test. Multiple linear regression including all parameters with P<0.1 was used for multivariate analyses. Kaplan–Meier estimates for outcome measures were made for the overall data and for strata- defined variables and were compared by means of the log-rank chi-square test. All reported P-values are 2-sided and P<0.05 was considered statistically significant.

Results

Baseline recipient and graft characteristics

Graft recipients (17 females/23 males) had type 1 diabetes for more than 20 years (IQR 21-33) with a median age of 46 years and BMI of 24 kg/m^2. They were all C-peptide negative (<0.09 ng/mL), had suboptimal glucose control before transplantation (median HbA1c of 7.7% (IQR 7.0–8.3]; 61 mmol/mol [53–67]) with large GV (46% [40-51]) and were using insulin at 0.57 units/kg/day [0.49 – 0.76].

The median donor and graft composition is shown in Table S2. Mean age, BMI and cold ischemia time were respectively 48 years, 25 kg/m^2, and 7.8 hours. The final grafts contained around 40% endocrine cells and a low percentage of damaged cells when observed under the electron microscopy.

Duration of graft function correlates to culture time of the first graft and recipient age

All patients achieved plasma C-peptide ≥0.5 ng/ml posttransplantation (PT) for a median of 51 months although duration of graft function varied considerably between recipients, illustrated by IQR between 14 and 90 months. A longer duration of C-peptide ≥0.5 ng/ml was noted in recipients (n = 10) who received a first graft that was composed from cell preparations that were all cultured for ≥96h (Figure 2A). Kaplan-Meier survival analysis shows that grafts of these 10 recipients had a half-life that was more than 80 months longer with the respect to the presence of C-peptide ≥0.5 ng/ml. In those 10 patients, mean duration of C-peptide ≥0.5 ng/ml was 103 months, compared to 48 months when not all cells were cultured for ≥96h (P=0.005).

In univariate and multivariate analysis, not only culture time of ≥96h, but also recipient age at transplantation correlated significantly with longer duration of C-peptide ≥0.5 ng/ml (Table 1). The youngest recipients (age <39 years) had a significantly shorter duration of graft function and insulin independence (Table 2).

Beta cell number (both per graft and total) and other baseline patient characteristics, such as BMI, HbA1c and immune parameters had no correlation to the duration of graft function.

Culture time of first graft and recipient age correlate with the duration of C-peptide ≥1.0 ng/ml and low glycemic variability and insulin independence

In total, 34 patients achieved C-peptide ≥1.0 ng/ml with low GV for a median duration of 22 months (IQR 7-27) and 24 patients achieved insulin independence for a median duration of 15 months (IQR 3.5-23).

Grafts following ≥ 96 h culture resulted in more patients with low GV and plasma C-peptide ≥1.0 ng/ml, both at PT-month 12 (9/10 vs. 12/30; P=0.005) and PT month 24 (7/10 vs. 6/30; P=0.007). In this subgroup, 9/10 patients became insulin-independent versus 15/30 in the other subgroup (P=0.03). The duration of C-peptide ≥1.0 ng/ml with low GV was more than

12 months longer in recipients of the ≥96h grafts (Figure 2B) while no difference was measured in duration of insulin independence (Figure 2C).

Recipients age <39 years showed shortest duration of C-peptide ≥1.0 ng/ml with low GV and insulin independence compared to recipients of 39 – 52 years and >52 years (Table 2).

Graft composition relates to culture time

To further understand the influence of culture time on the duration of graft function, actual graft composition and baseline characteristics were studied in function of culture times longer or shorter than 96 hours.

As expected, grafts composed from cells that were all cultured for more than 96 hours contained a lower total number of cells. The number of beta cells was also reduced but to a lesser extent. However, endocrine purity was significantly higher in long-term cultured grafts (P=0.028), illustrating that longer culture time results in grafts enriched with endocrine cells (Table 3). They also contained significantly less exocrine (*P*=0.03) and duct cells.

Recipients of grafts with a longer culture time had lower body weight and BMI than recipients of grafts with shorter culture times (Table 4). No differences in age and pretransplant HbA1c, autoimmune and alloimmune measurements were found.

Discussion

In this retrospective study, culture of islet preparations for at least 96h in first multidonor grafts was associated with prolonged graft survival. It also increased the duration of low glucose variability and a higher chance of reaching insulin-independence, which is compatible with obtaining a higher graft beta cell mass in the first months post transplantation.

To the best of our knowledge, this paper is the first to compare short (<96h) with long (≥96h) culture time of human islet cell preparations.

More patients transplanted with longer cultured cells achieved clinically relevant graft function and insulin independence and achieved better preserved graft function significantly longer than recipients of shorter cultured cells. Several mechanisms might explain these beneficial effects of prolonged culture.

Firstly, prolonged culture resulted in a reduced percentage of duct cells in the preparations. Human pancreatic duct cells are known to exert tissue-factor dependent procoaglulant activity[36] and to express CD40[37] which are both linked to instant blood mediated inflammatory responses and cell death.[38] In vitro human data from Ramnath et al suggest that short term culture already reduces the instant blood mediated inflammatory response after intraportal infusion,[39] and prolonging culture time even more might further improve these conditions.

Secondly, the lower number of exocrine cells and higher endocrine purity seen in preparations cultured ≥96h might further reduce their immunogenicity. Data in animal models showed a better survival of cultured islet transplants possibly linked to a higher percentage of endocrine cells in the graft and reduced immunogenicity.[31,40-43] Previous studies indicate that prolonged culture reduces the number of endothelial cells and white blood cells within graft[44-46] which may propagate islet transplant rejection.[46,47] Whatever the mechanisms involved, changes induced by long-term culture may be especially important in the first days and weeks of immune suppression. This study does not report on HLA antibody formation but our group previously reported on HLA sensitisation in patients with type 1 diabetes receiving islet cell grafts from multiple donors under the same immunosuppression as recipients in our current analysis.[48] HLA antibodies assessed by solid-phase flow-based Luminex method and complement-dependent cytotoxicity (CDC) assay were low in patients on immunosuppression. Allosensitization by Luminex but not CDC assay increased after

immunosuppression withdrawal and was correlated with lower beta cell purity but not with number of donors.[48]

No human data exist comparing transplantation of noncultured "fresh" islets with islet cells that have been cultured for a prolonged time. In this study, all preparations were cultured for at least 16 h, so that the comparison between fresh and prolonged culture in terms of transplant outcome was not possible. It cannot be excluded that the beneficial effects of higher beta cell mass and function in fresh preparations[49-52] outweighs the benefits of reduced procoagulant activity and immunogenicity of long cultured islet preparations.

Our data confirm the relationship between recipient age and achievement of good graft survival and insulin independence[3,13,53,54] and extend this finding to duration of these beneficial effects. It is well known in other organ transplantation settings that older age correlates with lower rates of rejection and improved graft survival.[55-57] Another reason for worse graft function in younger patients might be less compliance with the immunosuppressive regimen.[58,59] It is conceivable that combining younger recipient age with grafts that have higher immunogenicity maximizes the risk for acute rejection and therefore lower beta cell mass, resulting in shorter duration of graft function and insulin independence observed in our cohort. Currently, there are no established methods to diagnose rejection after islet transplantation, making proof of this hypothesis not feasible at this moment.

Our data also provide insight in the long term survival of 1 to 2 grafts transplanted within a 3 months period. We report a 10-year graft survival rate of 50% in recipients of long cultured islet grafts, which is in line with other centers.[60,61] However, without repeated infusions, no graft function is preserved longer than 15 year after the first 2 implants, in line with previously published registry data.[13] For that reason, our center recently started with reinfusions of islet grafts in patients that lost graft function several years after the first

implants. Future data will show if this approach results in restoring graft function with improvement of metabolic control beyond 15 years.

Our study has limitations, such as the small number of patients who received long-cultured preparations. It still remains to be determined, ideally in a prospective study, if a minimum culture time of 96 hours for the first graft is the most optimal when other culture conditions or methods are used. However, the scarcity of donor organs and the variability of isolation outcomes make such a prospective study very unlikely. Additional analysis in larger cohorts is also needed to determine the absolute of risk of allosensitization after exposure to multiple donor

In summary, human islet isolates with insufficient beta cell mass for implantation within 72 hours can be cultured for 96 hours and longer to combine preparations that together reach required dose and result in a metabolic benefit. The better outcome of grafts with a longer culture history was correlated to their higher endocrine purity, which is in line with observations in animal models.

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Figure Legends

Figure 1. Design of the study

*Number of cells and composition were determined from a combined sample before transplantation

The immune suppression regimen consisted of induction therapy with antithymocyte globulin (ATG-Fresenius, Fresenius, HemoCare, Redmond, WA) and maintenance therapy with mycophenolate mofetil (MMF,Cellcept, Roche, Basel, Switzerland) or mycophenolic acid (Myfortic, Novartis, Basel, Switzerland) and tacrolimus (Prograft, Astellas Pharma Europe, Staines, UK).

Figure 2. Graft survival curves.

Time to graft failure in recipients who received islet cells that were all cultured for at least 96 hours (n = 10, blue line) or not (n = 30, red line). The horizontal dotted line indicates 50% survival. The points on X-axis that intersect with the vertical dotted line indicate the duration that the endpoint was reached. Log-rank test was used for statistical significance.

Table 1. Factors correlated with duration of C-peptide ≥0.5 ng/ml

Parameters	Univariate		Multivariate	
	Estimate	P-value	Estimate	P-value
Graft characteristics				
Beta 1				
Beta Cell number (x10^6)	0.022	*0.891*		
Beta cell number /kg body weight (x10^6)	0.022	*0.866*		
Beta 2				
Beta Cell number (x10^6)	0.013	*0.994*		
Beta Cell number/kg BW (x10^6)	0.092	*0.630*		
Beta 1 + Beta 2				
Beta cell number (x10^6)	-0.068	*0.679*		
Beta cell number/kg BW (x10^6)	-0.034	*0.834*		
Culture time				
Beta 1				
All cells in culture ≥96 hours (yes/no)	**0.486**	***0.001***	**0.422**	***0.005***
Patient characteristics				
Age at transplantation	**0.313**	***0.049***	**0.307**	***0.036***
BMI (kg/m²)	**-0.273**	***0.088***		
HbA1c (%)	**-0.292**	***0.071***		
B-lymphocytes (CD19)	0.083	*0.611*		
T-lymphocytes (CD3)	-0.053	*0.733*		
Donor-specific HLA antibodies	-0.038	*0.815*		
Presence of auto-antibodies (yes/no)	-0.225	*0.162*		
T-cell autoreactivity for GAD (yes/no)	-0.232	*0.166*		
T-cell autoreactivity for IA2 (yes/no)	-0.107	*0.530*		
HLA A24 (yes/no)	-0.250	*0.120*		

Analysis of correlations was performed using the Pearson's rank correlation test. Multiple linear regression including all parameters with P <0.1 (in bold) was used for multivariate analyses.

Table 2. Median duration of 3 different outcomes according to age at transplantation

Median Age		Duration of C-peptide ≥0.5 ng/mL	Duration of C-peptide ≥1 ng/mL and GV <25%	Duration of Insulin independence
(Years)	n	(months)	(months)	(months)
< 39	9	36 (2-70)	5 (1-10)	1 (0-2)
39 -52	22	68 (46-92)	24 (15-33)**	12 (7-17)*
> 52	9	88 (51-125)*	18 (7-30)*	15 (0-31)

Data are mean (95% confidence interval). Statistical significance *P<0.05 and ** P< 0.005 compared with age <39 years

Table 3. Cell number and composition of grafts that contained cells that were all cultured for 96 hours or not

	First Graft		
	≧96h in culture	< 96 h	*P-value*
Number of recipients	10	30	
Beta cell per kg body weight (10^6/kg BW)	2.6 (2.1-3.1)	3.1 (2.7-3.5)	*0.187*
Graft cell number (x10^6)			
Total	**491** (392-591)	**757** (665-850)	***0.001***
Beta cells	**167** (135-200)	**208** (185-232)	***0.043***
Exocrine cells	**4** (0.2-7)	**40** (18-62)	***<0.001***
Duct cells	**205** (131-279)	**392** (323-461)	***0.001***
Damaged cells	49 (30-67)	69 (56-81)	*0.095*
Graft Composition (%)			
Endocrine	**49** (38-60)	**36** (31-41)	***0.028***
Exocrine	**0.7** (0.2-1.4)	**4.7** (2.4-7.0)	***0.003***
Duct	41 (31-50)	51 (46-55)	*0.058*
Damaged	10 (7-12)	9 (8-10)	*0.590*

Data are mean (95% Confidence Interval). Bold numbers indicate statistical significance (P<0.05).

Table 4. Baseline characteristics of recipients in function of culture time of first grafts

	All cells cultured ≥96 hours	< 100% cells cultured <96 hours
First Graft (n=40)	**10**	**30**
Body weight (kg)	62.4 ± 7.4	70.3 ± 8.4[a]
BMI (kg/m^2)	22.0 ± 1.9	24.3 ± 2.4[a]
Age at transplantation (years)	46.5 ± 6.4	45.4 ± 9.4
HbA1c (%)	7.2 ± 0.8	7.8 ± 0.9
Total lymphocyte count	1890 ± 996	1822 ± 500
CD3+ count	1384 ± 627	1259 ± 272
CD19+ count	296 ± 155	236 ± 98
Leucocyte count before ATG	5993 ± 2764	5972 ± 1438
CD4 /CD8 ratio	2.0 ± 0.7	2.0 ± 1.0
Auto-antibodies (ICA/GAD/IA2)	0/3/4	8/14/7
Positivity of 2 or more antibodies	4	18
T-cell autoreactivity against:		
IA2 and/or GAD (yes)	4/10	19/26
IA2 (yes)	1/10	10/26
GAD (yes)	2/10	16/26
Presence of donor-specific HLA antibodies	2/10	3/30
Second Graft (n=30)	**6/10**	**24 /30**
Total lymphocyte count	449.6 ± 165	661 ± 324
CD3+ count	201 ± 71	287 ± 163
CD19+ count	124 ± 67	216 ± 147
Leucocyte count	4583 ± 2254	3029 ± 1002
T-cell autoreactivity against:		
IA2 and/or GAD (yes)	4/6	14/24
IA2 (yes)	2/6	11/24
GAD (yes)	4/6	8/24
Presence of donor-specific HLA antibodies	3/6	8/23

[a]Statistical significance P<0.05 between 2 groups, independent T-test. Data are mean ± standard deviation.

Figure 1.

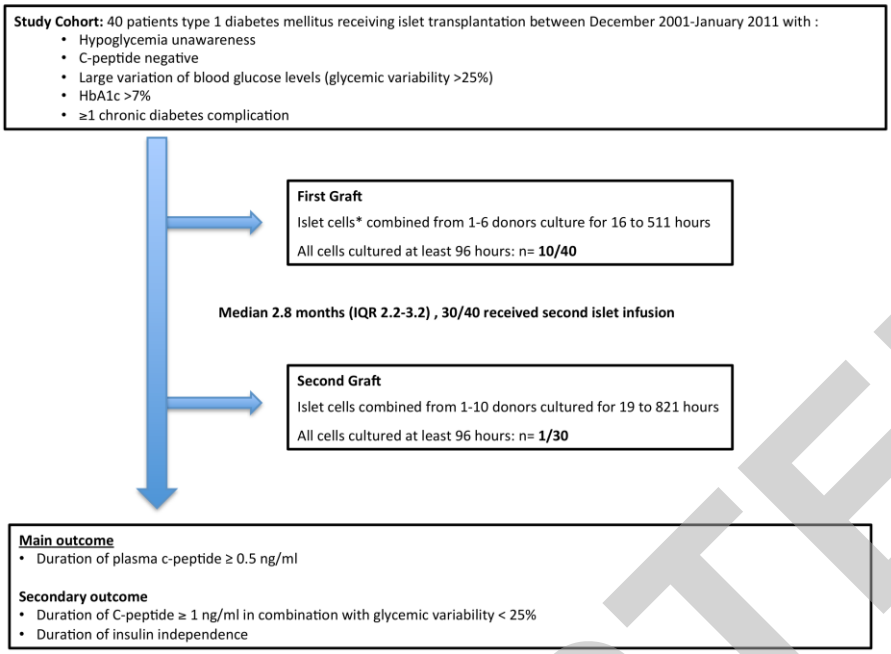

Figure 2.