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ORIGINAL ARTICLE

The impact of islet mass, number of transplants, and time between transplants on graft function in a national islet transplant program

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The UK islet allotransplant program is nationally funded to deliver one or two transplants over 12 months to individuals with type 1 diabetes and recurrent severe hypoglycemia. Analyses were undertaken 10 years after program inception to evaluate associations between transplanted mass; single versus two transplants; time between two transplants and graft survival (stimulated C-peptide >50 pmol/L) and function. In total, 84 islet transplant recipients were studied. Uninterrupted graft survival over 12 months was attained in 23 (68%) single and 47 (94%) (p = .002) two transplant recipients (separated by [median (IQR)] 6 (3–8) months). 64% recipients of one or two transplants with uninterrupted function at 12 months sustained graft function

Abbreviations: ATG, anti-thymocyte globulin; BMI, body mass index; CSII, continuous subcutaneous insulin infusion; DBD, donation after brain death; DCD, donation after cardiac death; DPP-IV, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; HLA, human leukocyte antigen; IAK, islet after kidney transplant; IEQ, islet equivalents; ITA, islet transplant alone; MDI, multiple daily injections; MMF, mycophenolate mofetil; MMTT, Mixed Meal Tolerance Test; NHS, National Health Service; NICE, National Institute for Health and Care Excellence.

at 6 years. Total transplanted mass was associated with Mixed Meal Tolerance Test stimulated C-peptide at 12 months ($p < .01$). Despite 1.9-fold greater transplanted mass in recipients of two versus one islet infusion (12 218 [9291–15 417] vs. 6442 [5156–7639] IEQ/kg; $p < .0001$), stimulated C-peptide was not significantly higher. Shorter time between transplants was associated with greater insulin dose reduction at 12 months (beta −0.35; $p = .02$). Graft survival over the first 12 months was greater in recipients of two versus one islet transplant in the UK program, although function at 1 and 6 years was comparable. Minimizing the interval between 2 islet infusions may maximize cumulative impact on graft function.

KEYWORDS
clinical research/practice, diabetes: type 1, endocrinology/diabetology, graft survival, islet isolation, islet transplantation

1 | INTRODUCTION

In type 1 diabetes, pancreatic beta-cell loss leads to insulin deficiency and an absolute requirement for insulin replacement.[1] Hypoglycemia is the most common side effect of insulin treatment with an average of 1–2 symptomatic episodes per week experienced by people with type 1 diabetes.[2,3] Recurrent hypoglycemia can lead to impaired awareness of hypoglycemia with an increased risk of severe hypoglycemia[4] defined as low blood glucose requiring external assistance, including help to administer carbohydrate, glucagon, or other resuscitating actions.[5] Severe hypoglycemia has an annual prevalence of 30%–40% in established type 1 diabetes[2,3] and is associated with increased morbidity and mortality.[6,7] Pancreatic islet allotransplantation offers the potential of stabilizing glycemic control, preventing recurrent severe hypoglycemia and restoring awareness of hypoglycemia.[8–12] Safety and efficacy have been demonstrated in long-term single site, multicenter Phase III and randomized controlled trials.[11–17] The primary indication for islet transplantation is C-peptide negative type 1 diabetes complicated by recurrent severe hypoglycemia requiring external assistance despite optimized medical management.[14,18,19]

The integrated UK islet transplant program was commissioned in 2008 and fully funded by the National Health Service (NHS) as a service for life-threatening recurrent severe hypoglycemia freely available at the point of care.[20] Assessment and transplantation are provided by seven geographically distributed islet transplant centers. Suitable deceased donor pancreata from throughout the UK are allocated to isolated islet and vascularized whole pancreas recipients on a common national waiting list. Following optimized standardized procurement by the National Organ Retrieval service, these are shipped to one of three islet isolation facilities with islets shipped to the transplanting center for infusion.[21]

For equitable sharing of available pancreata, and to achieve cost-effective clinical outcomes, the goal of delivering a total islet mass ≥10 000 IEQ/kg recipient body weight was implemented. Additional prioritization was given to recipients listed for a second transplant with the aim of achieving a median of 6 months between first and second transplant.[20] Insulin independence was not a primary aim with audit goals endorsed by the National Institute for Health and Care Excellence (NICE) including confirmation of graft function by measurement of stimulated C-peptide in a standardized Mixed Meal Tolerance Test (MMTT), resolution of recurrent severe hypoglycemia and attainment of HbA1c <7% (53 mmol/mol). These goals have subsequently been adopted internationally and are core to the recent consensus "Igls" criteria defining optimal function as HbA1c <7.0% without severe hypoglycemia, >50% reduction in insulin requirement and restoration of clinically significant C-peptide.[22] Graft survival (stimulated C-peptide >50 pmol/L) at a median of two years has been attained in 80% of UK recipients with effective prevention of further severe hypoglycemia.[20]

The aim of the current analysis was to compare graft function at 12 months in recipients of one versus two islet transplants and to explore associations with total transplanted islet mass and time between first and second transplant in those with uninterrupted graft function, in addition to longer term graft survival.

2 | MATERIALS AND METHODS

2.1 | Study design and participants

Recipients of a first islet transplant from the inception of the NHS islet transplant program in April 2008 until March 2015 at all centers (Bristol, Edinburgh, Kings College London, Manchester, Newcastle, Oxford and Royal Free London) were invited to take part in an ethically approved experimental medicine follow-up study following written informed consent.

The indication for islet transplantation was C-peptide negative type 1 diabetes complicated by recurrent severe hypoglycemia (≥2 events over the preceding 24 months)[5] despite optimized conventional management. Contraindications included total daily insulin

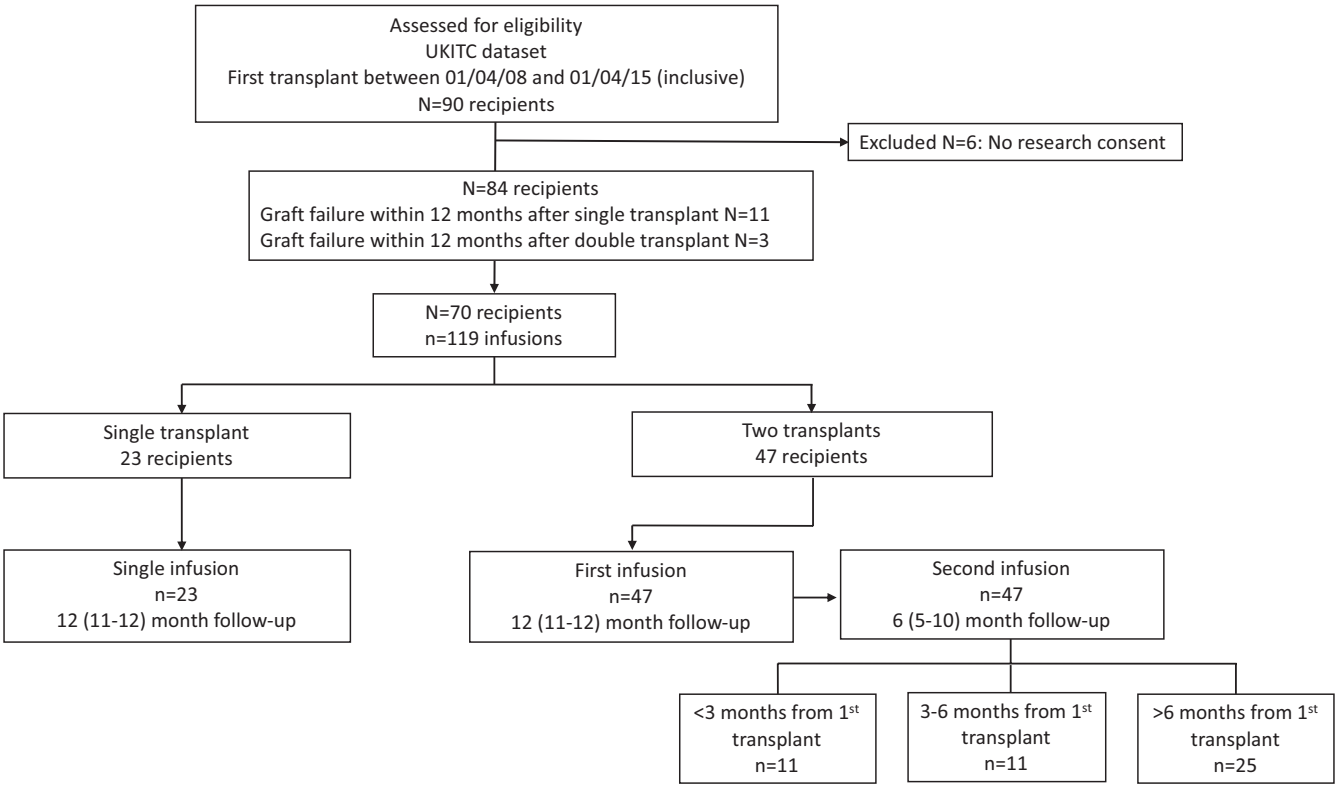

FIGURE 1 Numbers of participants in study that received one and two islet transplant infusions with functioning grafts and follow-up data

requirement ≥0.7 units/kg body weight and urinary albumin excretion rate >300 mg/24 h unless previous renal transplant.[20]

Participants consenting to research who had received one or two transplants over a 12-month period were studied. Comparative metabolic analysis at 12 months post-first transplant was undertaken in those with uninterrupted graft function with inclusion requiring an end-point MMTT at least 10 months post first transplant and 1 month post-second transplant (in recipients of two grafts). A Consort diagram including all UK islet transplant recipients over the study period is shown in Figure 1.

2.2 | Procedures

2.2.1 | Allocation of organs for transplant and release criteria

From 1st April 2008, pancreata donated after brain death (DBD) and after circulatory death (DCD) procured nationally were offered to the longest waiting ABO compatible recipient on the national waiting list following negative crossmatch.[21] Following implementation of the National Pancreas Allocation Scheme in November 2010, pancreata were offered initially to the highest priority suitable recipient on the joint vascularized pancreas/isolated islet waiting list with prioritization factors including waiting time, BMI (with higher BMI favoring offer to an islet recipient), HLA mismatch and recipient sensitization. In cases where adequate glycemic control was rapidly attained after a single islet transplant, a clinical decision was made not to relist for a second infusion. Otherwise, recipients were relisted after metabolic assessment at 1 month following first islet transplant, being provided with additional prioritization with the aim of providing a second graft at a median of 6 months after first graft.[21]

Standardized procurement of the pancreas, islet isolation protocols and shipment to transplant centers following 24-h culture have been reported previously.[23] Minimum product release criteria included islet mass ≥3000 IEQ/kg recipient body weight, viability >70% (nuclear exclusion dye staining) and purity >30% (dithizone staining). Participants were defined as having received transported islets if at least 1 of their islet preparations was isolated at a geographically distant UK facility and required transport to the transplant center where final viability was determined prior to transplantation.[23]

2.2.2 | Peri-operative patient management

All islets were transplanted by percutaneous transhepatic delivery into the portal vein under radiological guidance. Recipients received intravenous insulin and heparin peri-transplant followed by 7-day low molecular weight heparin.[18] Intensified insulin regimens

continued post-discharge with close follow-up by diabetes teams to help maintain optimal glycemic control.[19]

2.2.3 | Clinical review, metabolic assessment, and outcome measures

Clinical review was undertaken at 1, 3, 6, and 12 months after first graft along with a standardized MMTT with Ensure HP (6 ml/kg to a maximum of 360 ml consumed within 5 min, providing 1.1 Calories/ml; 23% fat, 55% carbohydrate and 22% protein).[24,25] All blood samples were centrifuged at 3000 rpm for 15 min at 4°C, separated and plasma frozen at −70°C until analysis.

Outcome measures were assessed at 12 months. Graft failure was defined as stimulated C-peptide <50 pmol/L with function evaluated using a range of validated parameters including fasting and 90-min MMTT stimulated glucose and C-peptide, beta-score,[24] BETA-2 score,[26,27] reduction in insulin dose and Igls criteria good β cell graft functional status—defined as HbA1c <7%, no severe hypoglycemia, insulin requirement <50% baseline and C-peptide positive.[22]

2.2.4 | Metabolic analyses

Glucose and C-peptide were analysed in a central reference laboratory. Glucose analysis was by Siemens Healthineers Dimension and C-peptide analysis by Perkin Elmer AutoDELFIA until December 2011 and Siemens Healthineers Immulite 2000 from December 2011, with quality-assured comparability of data.

2.3 | Data handling and statistical analysis

2.3.1 | Statistical analyses

In primary analyses at 12 months following first transplant, metabolic outcomes including: fasting and stimulated C-peptide, insulin dose reduction, beta,[24] BETA-2 scores,[26,27] and Igls criteria (good β cell graft functional status)[22] were assessed.

Outcomes in participants receiving two transplants were examined in relation to time between transplants: <3 months, 3–6 months and >6 months and compared to single transplant recipients by one-way ANOVA analyses with post-hoc testing.

The relationship between total islet mass transplanted and graft function at 12 months was explored in univariable linear regression models. In all recipients, the relationship between first islet transplant mass and metabolic outcomes at 12 months was also examined.

Multivariable linear regression models were constructed to determine differences in graft function comparing single versus double transplants after accounting for differences in transplanted islet mass, purity, viability, and donor age. Similar models were used to assess how time interval between two transplants, expressed continuously, was related to graft function. In secondary analyses, islets isolated from DBD versus DCD donors were compared.

All data sets were >95% complete and there was no data imputation. Data were expressed as mean (SEM) or median (IQR) as appropriate with $p < .05$ taken to denote statistical significance. Data were analyzed in STATA 15 (Stata Corporation).

3 | RESULTS

Eighty-four islet transplant recipients within the integrated NHS program participated in the study. Thirty-four received a single islet transplant and 50 received two grafts. Individuals with an episode of graft failure after first transplant were precluded from receiving a second graft. In others, a clinical decision not to relist for second transplant was made due to attainment of metabolic goals following single graft (stabilization of glucose variability and freedom from significant hypoglycemia with/without insulin independence).

Uninterrupted graft survival (defined as C-peptide >50 pmol/L) for 12 months following first islet transplant was attained in 23 (68%) single transplant recipients and 47 (94%) recipients of two grafts ($p = .002$).

Graft loss occurred at a median (range) of 132 (29–287) days post-single and at 321 (292–364) days in recipients of two sequential grafts ($p = .002$). Baseline parameters in those with graft loss over the first 12 months ($n = 14$) were comparable to those with uninterrupted graft function ($n = 70$) (Table S1).

Median (IQR) age at first transplant in participants with uninterrupted function was 51 (42–57) years and 70% were female. Prior to transplantation 54% were using continuous subcutaneous insulin infusion and 46% multiple-dose insulin injections (Table 1). 61 (87%) received islet transplant alone and 9 (13%) were islet after kidney (IAK) recipients.

The majority (62 [89%]) received alemtuzumab induction for the first transplant (Table 1). Maintenance immunosuppression was tacrolimus (Prograf: target trough level: 8–12 ng/ml)/mycophenolate mofetil (500 mg–2 g daily) in the majority (67 [96%]) with 5 (56%) IAK recipients taking additional low dose prednisolone before and after islet transplantation (Table 1).

Recipients with uninterrupted graft function over the first 12 months received a total of 117 islet infusions over the study period (Table 2). Donor age was 48 (42–53) years with BMI 29.4 kg/m^2. Ten (9%) of the transplants were from DCD donors. Median donor islet yield was 376 (310–500) × 10^3 islet equivalents (IEQ) with viability 90 (85–92)% and purity 80 (70–85)%. Isolation outcomes between DBD and DCD donors were comparable (Table S2; Figure S1).

Median islet mass per transplant was 5925 (4712–7633) IEQ/kg recipient body weight and donor/islet isolation parameters were comparable between preparations used for single transplants, those for the first of two infusions and those for the second of two infusions (Table 2; Figure S1). Overall, 46% of islet transplants were carried out with transported preparations with no significant difference in proportions transported between the groups ($p = .79$). Only islet

TABLE 1 Recipient demographics, insulin delivery modality, induction, and maintenance immunosuppression

	All $N = 70$	Single transplant $N = 23$	Two transplants $N = 47$	p
Age (years)	51 (42–57)	54 (43–64)	50 (42–56)	.17
Female (%)	70	88	65	.27
CSII: MDI *n* (% CSII)	38: 32 (54)	12: 11 (52)	26: 21 (55)	.80
ITA: IAK (*n*)	61:9	20:3	41:6	.40
ITA (%)	87	87	87	
Induction (first transplant) Alemtuzumab: ATG: Daclizumab: Basiliximab (*n*)	62:2:2:4	21:1:1:0	41:1:1:4	.77
Tacrolimus/MMF ± prednisolone (all *n*)	67	23	44	.17
Other immunosuppression regimen[a]	3	0	3	

Note: Data are median (IQR), number (%).

p, one versus two transplants (unpaired *t*-test).

Abbreviations: ATG, anti-thymocyte globulin; CSII, continuous subcutaneous insulin infusion; IAK, islet after kidney; ITA, islet transplant alone; MDI, multiple daily injections; MMF, mycophenolate mofetil.

[a]Other regimens: tacrolimus/sirolimus; tacrolimus/azathioprine and; cyclosporin/mycophenolate mofetil—each in a single recipient.

TABLE 2 Donor anthropometry and islet isolation data

	All infusions ($N = 117$)	Single infusion in 23 recipients ($N = 23$)	Two infusions in 47 recipients ($N = 94$)		p
			First infusion $N = 47$	Second infusion $N = 47$	
Donor age	48 (42–53)	48 (41–55)	47 (38–55)	49 (44–52)	.99
Male sex (%)	47	39	55	29	.33
Donor height (cm)	169 (161–176)	168 (160–177)	173 (163–178)	169 (164–176)	.28
Donor weight (kg)	80 (75–90)	80.0 (70.0–90.0)	80 (75–90)	85 (78–95)	.58
Donor BMI (kg/m^2)	29.4 (26.3–32.5)	29.3 (24.1–33.0)	28.4 (26.3–31.6)	29.9 (26.1–33.0)	.45
DCD/DBD	10/107	2/21	8/39	2/45	.12
Islet yield IEQ (×10^3)	376 (310–500)	393 (335–550)	374 (305–500)	376 (300–481)	.66
Islet viability (%)	90 (85–92)	85 (82–90)	90 (85–91.5)	90 (85–94)	.03
Islet purity (%)	80 (70–85)	80 (65–85)	75 (69–90)	80 (73–90)	.37
IEQ/kg recipient body weight	5925 (4712–7633)	6442 (5156–7639)	5897 (4409–6992)	5788 (4461–7717)	.44
Shipped before transplantation, *n* (%)	54 (46)	12 (52)	20 (42)	22 (47)	.79

Note: Data are median (IQR), number (proportion). *p*, islet preparations from single infusions versus first of two infusions versus second of two compared by one-way ANOVA.

viability for single grafts (median 85%) was statistically lower than the viability of both first and second preparations (90%) in those receiving two transplants (Table 2).

3.1 | Comparison of metabolic outcomes at 12 months in one versus two transplants

Recipients received a total islet mass of between 4030 and 21 722 IEQ/kg. Although first transplant mass was comparable in those receiving a single transplant (6442 [5156–7639] IEQ/kg) versus those receiving two transplants (5897 [4409–6992] IEQ/kg, $p = .44$), those receiving two transplants received 1.9-fold greater total islet mass compared with solitary transplant recipients (two transplants: 12 218 [9291–15 417] versus solitary transplant 6442 [5156–7639] IEQ/kg, $p < .0001$) (Table 4).

Outcomes were assessed at 12 (11–12) months post-first transplant (in recipients of one and two transplants) and 6 (5–10) months post-second transplant when received. Body weight was lower at 12 months compared with baseline (Table 3). In the overall cohort, 29 participants were taking GLP-1 receptor agonists and/or DPPIV inhibitors (11 (48%) solitary transplant and 18 (38%) recipients of 2 infusions). When the analyses were run including only participants not on these agents, weight reduction post-transplant remained significant ($p = .04$).

TABLE 3 Anthropometric and metabolic parameters pre-transplant and at 12 months post-first islet transplant

	Pre-transplant All recipients $n = 70$	Post-transplant All recipients $n = 70$	p	Single infusion[a] $n = 23$			Two infusions[b] $n = 47$				
				Pre-transplant	Post-transplant	p	Pre-transplant	Post-transplant	p	$p1$	$p2$
BMI (kg/m^2)	24.8 (22.7–27.5)	23.0 (21.2–26.0)	<.001	24.9 (23.4–27.7)	23.5 (21.4–27.0)	.07	24.8 (21.4–27.5)	22.8 (20.6–26.0)	<.001	.51	.42
Weight (kg)	66.0 (60.0–75.0)	62.7 (56.2–73.2)	<.001	65.0 (61.0–72.8)	63.2 (56.2–73.8)	.04	66.2 (58.8–79.3)	61.8 (56.1–73.2)	<.001	.92	.78
24 h insulin dose (units)	34 (24–42)	16 (6–26)	<.001	31 (22–40)	19 (9–27)	<.001	35 (25–42)	14 (6–25)	<.001	.58	.82
Insulin dose (U/kg)	0.51 (0.36–0.60)	0.24 (0.09–0.39)	<.001	0.50 (0.33–0.56)	0.24 (0.16–0.39)	<.001	0.52 (0.36–0.62)	0.24 (0.08–0.37)	<.001	.47	.92
HbA1c (%)	7.9 (7.2–9.5)	6.5 (6.0–7.2)	<.001	7.9 (7.1–9.0)	6.6 (6.0–7.2)	<.001	7.9 (7.2–9.5)	6.5 (6.0–7.1)	<.001	.79	.46
HbA1c (mmol/mol)	63 (55–80)	48 (42–55)	<.001	63 (54–75)	49 (42–55)	<.001	63 (55–80)	47 (42–54)	<.001	.79	.46

Note: Data are median (IQR).

p, pre- versus post-transplant values in individual recipients.

p1, recipients of one versus two transplants (pre-transplant values).

p2, recipients of one versus two transplants (post-transplant values).

[a]Represents pre- and post-transplant data from recipients receiving a single transplant.

[b]Represents pre- and post-transplant data from recipients receiving two transplants.

Total daily insulin dose was reduced by 50% compared with pre-transplant accompanied by a significant reduction in HbA1c from 7.9 (7.2–9.5)% to 6.5 (6.0–7.2)% ($p < .001$). All changes from pre-transplant values were comparable in recipients of one versus two islet transplants with no statistical differences between groups at baseline or at 12 months (Table 3). The comparable metabolic benefits following one or two transplants were unaffected by the exclusion of the 13% IAK recipients in both groups.

Although recipients of two transplants received a much greater total islet mass, only fasting C-peptide and accompanying C-peptide: glucose ratio were higher at 12 months with no significant difference in other measures of graft function including the beta- and BETA-2 score and Igls criteria based on good beta-cell graft functional status (Table 4). A period of insulin independence was achieved in 17% of recipients in both groups.

Two (9%) single transplant recipients received DCD islets with 8 (17%) recipients of two grafts having a DCD donor for one transplant. Outcomes following DCD transplant were comparable to DBD (Table S3).

Median follow-up in recipients with maintained function over the first 12 months was 38 (27–52) months. Kaplan–Meier survival curves showed maintained graft function at 6 years in 64% of recipients of one or two grafts (Figure 2).

In univariable linear regression analyses including all transplant recipients, total islet mass (IEQ/kg) transplanted was positively and significantly associated with 12-month MMTT fasting and 90-min C-peptide, 90-min C-peptide: glucose ratio and BETA-2 composite scores but not HbA1c, insulin dose reduction, beta-score or the Igls criteria for good β cell graft functional status (Table S4). In solitary transplant recipients, total transplanted mass was positively and significantly associated with the 90-min C-peptide and 90-min C-peptide: glucose ratio (Table S4). In participants receiving two islet transplants, total transplanted mass was significantly associated with 90-min C-peptide, 0 and 90-min C-peptide: glucose ratio, beta and BETA-2 scores and the Igls criteria (Table S4). There was no significant difference in beta scores between the groups: one transplant alone versus two transplants in <3 months, two transplants between 3 to 6 months and two transplants between 6 and 12 months of each other (Figure S2).

The slope of the line relating islet mass transplanted and stimulated C-peptide was shallower in recipients of two versus a single transplant (Figure 3).

In multivariable linear regression models adjusted for confounders (islet number, purity, viability, and donor age), there were no statistically significant differences in 90-min MMTT C-peptide, 90-min C-peptide: glucose concentrations, beta and BETA-2 scores in recipients of one versus two grafts at 12 months post-transplant; however Igls criteria for good β cell graft functional status was superior in recipients of one graft at 12 months when comparing recipients of one versus two transplants (Table S5). Further multivariable linear regression models were run adjusting for the above confounders as well as the presence of GLP-1 agonists and, or, DPP-IV inhibitors. The statistically significant difference in Igls criteria for good β cell

TABLE 4 Graft function at 12 months comparing one versus two transplants and time interval between two transplants

Metabolic outcome	All recipients $N = 70$ recipients	one transplant $N = 23$ recipients[#]	two transplants $N = 47$ recipients	p	<3 months $N = 11$ recipients[#]	3–6 months $N = 11$ recipients[#]	>6 months $N = 25$ recipients[#]	p^*	p^{**}
Total Islet mass transplanted/kg recipient body weight	10 160.5 (7143–13 711)	6442 (5156–7639)	12 218 (9291–15 417)	<.0001	12 218 (9253–15 496)	8970 (7120–10 325)	13 579 (11 254–15 441)	<.0001[a,c]	.01[f]
0 min glucose (mmol/L)	7.6 (6.6–10.0)	7.1 (6.3–8.7)	7.8 (6.8–10.4)	.13	7.6 (6.9–10.2)	9.1 (6.9–15.4)	7.7 (6.6–12.7)	.21	.33
0 min C-peptide (pmol/L)	226 (89–421)	89 (54–308)	270 (190–230)	.006	260 (174–403)	421 (219–525)	271 (213–410)	.04	.54
0 min C-peptide: glucose	29 (13–56)	15 (8–45)	31 (18–37)	.03	27 (17–70)	31 (14–66)	39 (22–57)	.30	.98
90 min glucose (mmol/L)	14.6 (10.6–17.9)	16.1 (11.4–19.0)	13.9 (10.7–17.5)	.21	13.1 (9.7–17)	16 (12.1–20.9)	13.7 (10.6–16.6)	.25	.28
90 min C-peptide (pmol/L)	527 (273–884)	440 (168–858)	574 (342–903)	.12	688 (182–861)	544 (334–884)	533 (357–1033)	.40	.28
90 min C-peptide: glucose	36 (16–75)	31 (11–66)	41 (21–75)	.19	48 (10–115)	32 (16–45)	44 (22–104)	.41	.54
Beta score	4 (2–5)	4 (2–5)	3 (2–5)	.95	3 (2–5)	3 (2–4)	4 (2–6)	.65	.49
BETA-2 score	7 (4–13)	5 (4–11)	9 (5–14)	.46	9 (3–16)	5 (4–6)	11 (6–15)	.17	.10
Insulin reduction (%)	48 (26–80)	41 (17–78)	55 (30–80)	.23	75 (17–90)	48 (32–87)	48 (27–68)	.28	.24
Insulin independence (%)	17	17	17	.86	18	27	8	.50	.31
Igls criteria (%)[†]	67	74	64	.54	64	36	76	.08	.07

Note: Data are median (IQR).

p, outcomes in recipients of one versus two transplants.

p^*, ANOVA with post hoc testing between transplant recipients in all four groups[#] receiving one or two transplants.

p^{**}, ANOVA with post hoc testing between transplant recipients in the groups receiving 2 transplants.

a, 1Tx versus ×2Tx <3 months; b, 1Tx versus ×2Tx 3–6 months; c, 1Tx versus ×2Tx >6 months; d, ×2Tx <3 months versus 3–6 months; e, ×2Tx <3 months versus >6 months; f, ×2Tx 3–6 months versus >6 months.

†Igls criteria—good β cell graft functional status used.

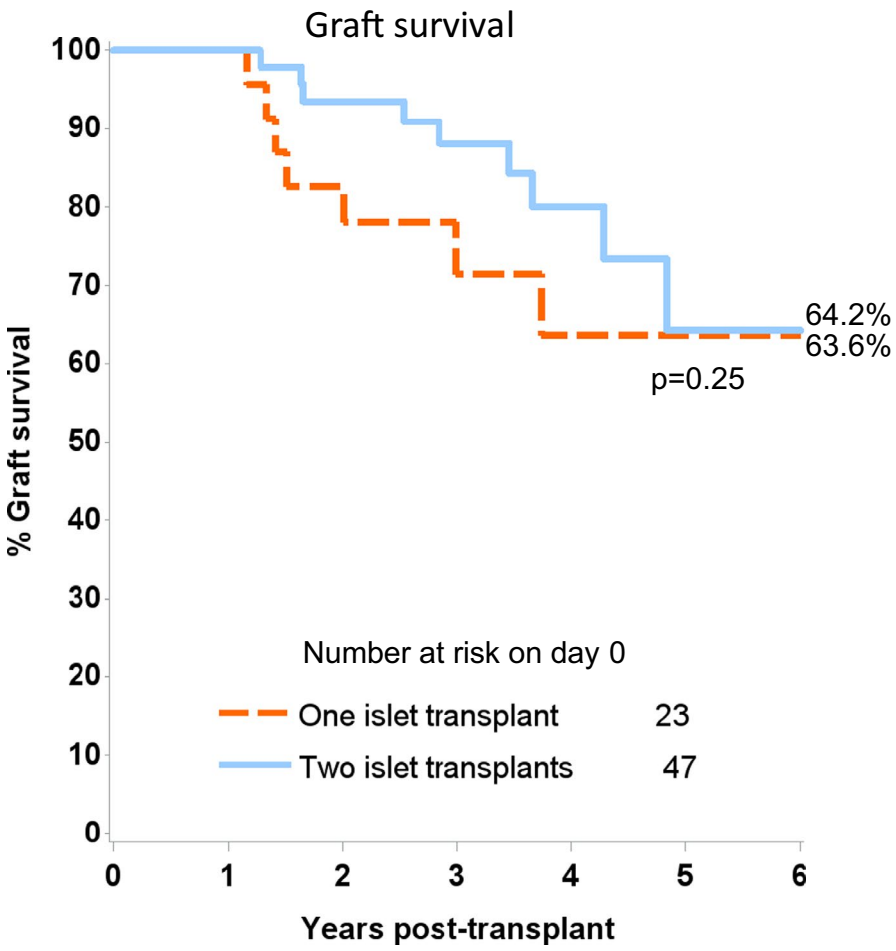

FIGURE 2 Kaplan–Meier survival curve demonstrating graft survival over 6 years follow-up after one (orange) versus two (blue) islet transplants. Recipients were included if the graft was still functioning at 12 months post-transplant

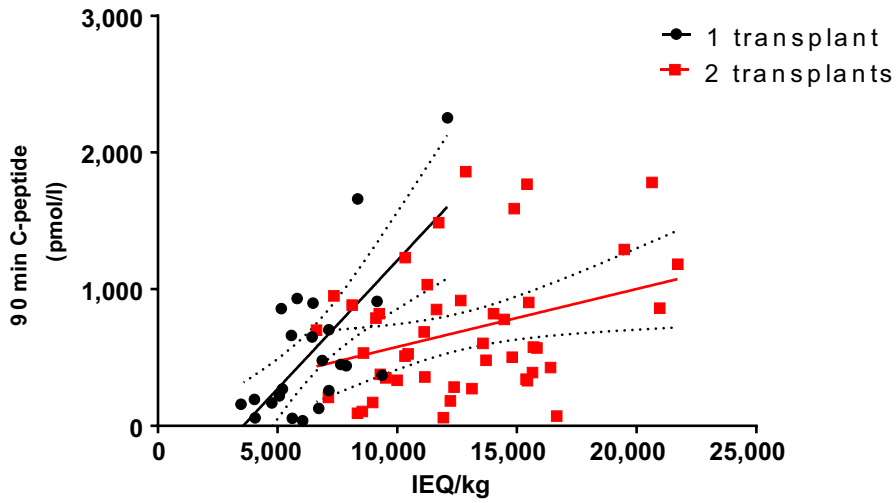

FIGURE 3 Total transplanted islet mass in recipients of one (black) versus two (red) transplants and corresponding MMTT 90-min C-peptide measured at 12 months post first transplant. The slope of the line relating transplanted islet mass in two versus one transplant with 90-min C-peptide was shallower ($p = .26$)

graft functional status in those receiving one versus two grafts remained ($p = .03$; Table S5).

In secondary analyses transplant recipients receiving islets from DBD versus DCD donors were compared in recipients of one and two transplants; no recipient who received two transplants received these from two DCD donors. Although the numbers of DCD donors were small, the 12-month metabolic outcome results did not show statistically significant differences based on whether recipients received islets from DBD versus DCD donors (Table S2).

3.2 | Impact of time between transplants

The median time between first and second transplants was 6 (3–8) months. Recipients of two transplants undertaken within 3 months ($n = 11$) were compared to those re-transplanted between 3–6 months ($n = 11$) and >6 months after first transplant ($n = 25$). Those re-transplanted after 3–6 months received a lower total islet mass versus the other two groups (Table 4). All metabolic outcome measures were comparable between the three groups in unadjusted

analyses with the exception of a 75% reduction in total daily insulin dose in those re-transplanted within 3 months compared to a 48% reduction in the other groups (Table 4). In multivariable-adjusted linear regression models, adjusting for islet number, purity, viability and donor age, a shorter time interval between transplants was significantly associated with greater insulin dose reduction ($p = .02$; Table S6).

4 | DISCUSSION

Islet transplantation is adopted in the UK as a standard of care for type 1 diabetes complicated by recurrent severe hypoglycemia despite optimized conventional therapy. Prevention of further severe hypoglycemia has been attained, without sustained insulin independence in the majority. We evaluated the associations between transplanted islet mass, number and timing of transplants and graft function.

Graft failure rate over the first year was higher in single transplant recipients but it is important to recognize that UK patients can only receive a priority second graft while the first transplant is functioning, positively biasing graft survival towards the two transplant group.

Following exclusion of those with graft failure, graft function determined by Mixed Meal Tolerance Test parameters and composite beta-cell function scores at 12 months following two sequential transplants was remarkably comparable to that in single transplant recipients despite receiving an almost 2-fold greater total islet mass. Only fasting C-peptide was significantly higher in recipients of two transplants. HbA1c and insulin dose reduction were comparable with insulin independence attained in 17% in both groups. Optimal or good function determined by Igls criteria was achieved at 12 months in three-quarters of single graft recipients versus two-thirds of two graft recipients. Regression analysis supported a positive association of single graft with favorable Igls score.

Longer-term follow-up demonstrated ongoing graft function at 6 years in two-thirds of recipients of one or two grafts with uninterrupted function over the first year. This confirms that, as currently configured, the UK program has high graft survival rates and that sustained graft function over the first year is a good predictor of long-term function with low attrition rates.

In a linear regression analysis, total islet mass infused corrected for recipient body weight was associated with greater graft function. In previous studies including retrospective analysis within the international Collaborative Islet Transplant Registry, the overall mass has been the only islet product parameter associated with clinical outcomes.[9,28] In these studies, however, associations have been with the attainment of insulin independence without statistically significant associations with continuous C-peptide measures of graft function.

Interestingly, the mass of the first transplant was associated with graft function in those receiving one and two transplants. Although requiring larger scale confirmation, this is in keeping with current practice within the UK and other programs internationally of aiming for a higher mass in first versus subsequent transplants.[20]

Although the association between greater transplanted mass and better graft function was seen in recipients of one and two transplants, the slope of the relationship between islet mass transplanted and stimulated C-peptide was shallower in recipients of two transplants. This could be explained by several factors including firstly selection effects whereby: (i) recipients of well-functioning single grafts were not listed for second grafts and (ii) recipients of poorly functioning single grafts were listed for second grafts and secondly that late second transplants (median delay 6 months) might not provide the expected improvements in overall graft function. Overall, there is a synergistic impact of the second transplant in terms of delivering insulin independence which is attenuated by the median delay of 6 months between infusions.

The median total transplanted islet mass was >10 000 IEQ/kg in recipients of two grafts. The potential for delivering insulin independence following transplantation of >10 000 IEQ/kg was demonstrated in the seminal Edmonton series and has been replicated in several studies.[8,12,13,16,17] Importantly, each of these studies was set up with insulin independence as a primary goal with rapid sequential transplantation until this was achieved. In the Lille program, delivery of two or three transplants within 67 days led to maintained insulin independence in 57% of recipients at more than 3 years after transplantation.[29]

The UK Pancreas Allocation Scheme has delivered its target interval of a median of 6 months between grafts in those receiving routine followed by priority transplants. In the minority receiving a second transplant within 3 months, there was a 75% reduction in exogenous insulin dose and multivariable-adjusted analyses correcting for confounding variables showed greater insulin dose reduction with shorter time to second graft. As a result of this analysis, the Allocation Scheme has been revised toward a median of 3 months between transplants and all centers have recently committed to a revised policy of rapid relisting for all recipients without early single graft insulin independence.

Preferential allocation of higher BMI donors to isolated islet recipients within the UK Pancreas Allocation Scheme has been associated with higher donor age with 45 (38%) of transplanted preparations within the current analysis isolated from donors aged above 50 years, previously associated with less good islet function *in vitro*, *in vivo* in diabetic mice and in a clinical cohort receiving a single islet transplant.[30] Nevertheless, the current analysis which includes 10 (9%) DCD pancreata with comparable outcomes to DBD transplants has reconfirmed the potential for attaining clinical and metabolic goals in a unified solid organ pancreas and isolated islet transplant program providing access to all potential donors procured nationally.

It is of course possible that factors other than the time interval between transplants has relatively favored outcomes in those chosen to have a single transplant and limited graft function in recipients of two transplants. Comparable 12 month and long-term outcomes after one and two transplants must be considered in the context of

excluding those with early graft failure over the first year—affecting a higher proportion of those receiving a single graft. Although not formally analyzed in the current study, *de novo* donor-specific antibody formation was not common in this cohort (particularly following exclusion of those with early graft loss). Based on our clinical knowledge of our patients, alloantibody sensitization or any other intercurrent pathology was not a significant factor delaying access to a second graft, evidencing the effectiveness of the UK Pancreas Allocation Scheme in providing access to suitable donors and delivering a median interval between grafts of 6 months by design. Although there were more DCD pancreases in the first graft of two transplants versus a single transplant (21% vs. 10%), these were carefully selected with comparable islet isolation outcomes to DBD organs.

The strengths of the study include rigorous standardized metabolic follow-up including Mixed Meal Tolerance Test at 12 months with centralized quality-assured C-peptide analysis for all participants within a research protocol including all centers in a unified national program. An *a priori* plan to exclude the small minority of recipients with graft failure from the analysis enhances the potential for undiluted assessment of the impact of transplanted islet mass, number of transplants and interval between transplants on functional islet mass at 1 year. Nevertheless, potential causes of graft loss were not included in the current analysis. Other weaknesses include some variability in induction and immunosuppression regimens but with well-matched groups for all of the comparative analyses performed. Numbers receiving the second transplant within three months were relatively small and, although greater insulin dose reduction in this group supports the association of longer interval between transplants with a continued need for exogenous insulin, differences between groups were not statistically significant.

In conclusion, we have demonstrated that graft function sufficient to restore metabolic control has been comparably achieved and sustained with one or two transplants in an integrated nationally funded program where the decision of whether to proceed to a second transplant was at the discretion of the recipient clinical team without the attainment of insulin independence as a primary goal. This approach has been facilitated by an equitable sharing scheme between vascularized pancreas and isolated islet recipients including extended criteria donors and with a maximum of two transplants per recipient. The current analysis supports the provision of two rather than a single transplant to islet recipients in view of lower failure rate over the first year. Furthermore, it supports a further iteration of the program towards listing for priority second transplantation as soon as a primary function of the first graft has been confirmed in parallel with a change in the allocation scheme to maximize the number of re-transplants within 3 months. These changes have recently been implemented with the goal of truly optimizing maximal graft function evidenced by higher rates of sustained insulin independence.

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SF contributed to study design and performed all data analysis, interpreted data and wrote the first draft of the manuscript; AF contributed to data collection and manuscript drafting. JAMS contributed to study design and data interpretation. CC undertook longer term graft survival analysis. The study was interpreted by all authors and all authors commented on and approved the final version of the manuscript. SF is the guarantor of this work and, as such, had full access to all study data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The authors thank the Study Manager Cath Brennand and Data Manager Ruth Wood, in addition to the many surgeons, physicians, research nurses, transplant coordinators, and clinical research associates at all sites who contributed to data collection. Donor data were obtained from the UK Transplant Registry and analysed by NHSBT Statistics and Clinical Audit, Bristol, UK. C-peptide and glucose assays were performed by the NIHR Cambridge Biomedical Research Centre, Core Biochemical Assay Laboratory. The UK islet transplant program is funded by the National Health Service National Commissioning Group. The current study was funded by the Diabetes UK Grant: Biomedical and Psychosocial Outcomes of Islet Transplantation BDA 06/0003362.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in NHSBT at https://tinyurl.com/2a7wv44u

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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