De Novo Donor Specific HLA Antibodies after Combined Intestinal and Vascularised Composite Allotransplantation

Annemarie Weissenbacher, MD[1, 2*], Georgios Vrakas, MD, PhD[1*], Mian Chen BMed, MSc[1], Srikanth Reddy, MBBS[1], Philip Allan, MBBS[1, 3], Henk Giele, MD[4], Martin CNM Barnardo, PhD[1], Anil Vaidya, MD[5], Peter J Friend, MBBS, MD[1], Susan V Fuggle, DPhil[1]

1. Oxford Transplant Centre, Oxford University Hospitals, Churchill Hospital, Old Road, Oxford, OX3 7LE, UK
2. Department for Visceral-, Thoracic and Transplant Surgery, Innsbruck Medical University, Innsbruck, Austria
3. Translational Gastroenterology Unit, John Radcliffe Hospital, Headley Way, Oxford, OX3 9DU, UK
4. Department of Plastic and Reconstructive Surgery, John Radcliffe Hospital, Headley Way, Oxford, OX3 9DU, UK
5. Apollo Hospitals Enterprise, Transplant Department, 21 Greams Road, Chennai, 600006, India

*Georgios Vrakas and Annemarie Weissenbacher contributed equally as the first authors

AW, GV, MC, PF, SF: Participated in the research design, writing the paper, performing the research and in analyzing the data.

SR: Participated in the research design, writing the paper and in performing the research.

PA, HG, MCNMB, AV: Participated in writing the paper and in performing the research.

Corresponding author: Georgios Vrakas, +447902580316, Oxford Transplant Centre, Churchill Hospital, Old Road, Oxford, OX3 7LE, UK, georgios.vrakas@ouh.nhs.uk

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Abbreviations

AWTx	Abdominal Wall Transplantation
CDC	Complement Dependent Cytotoxicity
CIT	Cold Ischemia Time
CMV	Cytomegalovirus
cRF	Calculated HLA Antibody Reaction Frequency
dnDSA	De Novo Donor Specific HLA Antibodies
DSA	Donor Specific HLA Antibodies
EBV	Epstein-Barr Virus
EDTA	Ethylene-diamine-tetra-acetic acid
ITx	Intestinal Transplantation
IV	Intravenous
MFI	Median Fluorescence Intensity
MMVTx	Modified Multivisceral Transplantation

PCR-SSP	Polymerase Chain Reaction- Sequence Specific Primer
SBTx	Small Bowel Transplantation
VCA	Vascularised Composite Allotransplantation/Allograft

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Abstract

Combining vascularised composite allotransplantation (VCA) with intestinal transplantation to achieve primary abdominal closure has become a feasible procedure. Besides facilitating closure, the abdominal wall can be used to monitor intestinal rejection. As the inclusion of a VCA raises the possibility of an enhanced alloimmune response, we investigated the incidence and clinical effect of de novo donor specific HLA antibodies (dnDSA) in a cohort of patients receiving an intestinal transplant with or without a VCA. The sequential clinical study includes 32 recipients of deceased donor intestinal and VCA transplants performed between 2008 and 2015; 8 (25%) modified-multivisceral transplants and 24 (75%) isolated small bowel transplants. A VCA was used in 18 (56.3%) cases. There were no episodes of intestinal rejection without VCA rejection. Fourteen patients (14/29; 48.3%) developed dnDSA. In the VCA group, fewer patients developed dnDSA; 6/16 (37.5%) VCA vs. 8/13 (61.5%) non-VCA. There was no statistically significant difference in one and three-year overall graft survival stratified for the presence of dnDSA; p=0.286. In the study there is no evidence that the addition of a VCA increases the incidence of dnDSA formation compared to transplantation of the intestine alone.

Introduction

The field of intestinal transplantation (ITx) is small with less than 200 transplants having been performed in the UK and less than 3000 worldwide [1, 2]. There is even less international experience of vascularised composite allotransplantation (VCA), which is the youngest field in transplantation, but one that has expanded the scope of reconstructive surgery. In the early days of transplantation, while VCA was viewed as a clinical option, the known high antigenicity of skin combined with evidence from experimental models suggested that the risk of rejection was too high to be translated into clinical practice [3]. Similar to ITx, reconstructive transplantation has evolved from being considered high risk to become a clinical reality in the past two decades. However, both functional and immunological outcomes have exceeded initial expectations, and for carefully selected patients, VCA is now seen as the best restorative option available.

Intestinal transplantation and VCA developed independently over the last 20 years, until Levi et al. [4] published “Transplantation of the abdominal wall” in Lancet in 2003. They proposed abdominal wall transplantation (AWTx) as a solution for the problems of abdominal wall closure after ITx when the abdominal space is often severely contracted and the skin scarred due to multiple operations prior to the transplant [5, 6].

Intestinal and composite transplants have reached a stage at which the investigation of long-term graft outcomes and immunological responses is becoming essential. A humoral immune response may be detrimental to a graft, leading to functional deterioration and the requirement for regular clinical follow up and/or anti-rejection treatment. Transplants may be

performed in the presence of pre-existing donor specific HLA antibodies (DSA) in previously sensitized intestinal and VCA recipients (preformed DSA). Alternatively DSA may develop after transplantation (de novo DSA; dnDSA) [7- 10].

The effects of DSA/dnDSA on intestinal and vascularised composite allografts are largely unknown, but the majority of studies have reported that the presence of DSA is associated with rejection and graft impairment [10, 11]. Even less is known of the occurrence and impact of dnDSA in combined ITx and VCA patients; especially whether a VCA, a graft with potentially high immunogenicity, increases the development of dnDSA. We have therefore investigated our cohort of recipients receiving an ITx graft (isolated small bowel transplant (SBTx) or modified multivisceral transplant (MMVTx)) performed with or without a full-thickness VCA to determine whether the addition of a VCA increased the incidence of dnDSA following transplantion and whether the development of dnDSA had an impact on graft survival.

Patients and Methods

This is a retrospective study of 32 consecutive deceased donor intestinal transplants performed with or without a VCA at the Oxford Transplant Centre between October 2008 and December 2015. The combined intestinal and abdominal wall transplant program is approved under the licence #40038 HTA (Human Tissue Authority):00041. Outcome variables were evaluated in all ITx [small bowel (SBTx) and modified-multivisceral (MMVTx)] and combined ITx+VCA transplants. The MMVTx comprised stomach, pancreaticoduodenal-complex, small bowel and the right hemicolon. None of the transplants included a liver allograft. The endpoints of this study were the development of dnDSA after transplantation and graft survival at one and three years after transplantation.

Immunosuppression

All recipients, intestinal transplant +/- VCA, received lymphocyte-depleting induction therapy with the CD52 antibody alemtuzumab (Campath®, 30mg intravenous (iv) within 6 hours of reperfusion and a second dose at 24 hours), followed by tacrolimus monotherapy for maintenance immunosuppression. We aimed for tacrolimus trough levels of 10-12 ng/ml in the first 6 months and for 8-10 ng/ml thereafter.

Infection prophylaxis

Meropenem (500mg TDS, iv) and micafungin (100mg OD, iv) were given for 5 days. Cytomegalovirus (CMV) prophylaxis was administered in cases where either the donor or the recipient was CMV positive. It comprised iv ganciclovir

(5mg/kg OD, iv), followed by valganciclovir (900m OD, po) for 1 year. No CMV prophylaxis was used when both donor and recipient were negative for CMV IgG.

Clinical follow-up protocol

Intestine: The endoscopy and biopsy protocol for the clinical follow-up was the same in both groups. It comprised protocol- and indication-driven zoom endoscopies and mucosal biopsies. Protocol biopsies were performed twice a week for the first 3 months and per indication thereafter (deterioration of bowel function/ suspicion of rejection). Biopsies were reviewed according to the grading system presented at the Eighth International Small Bowel Transplant Symposium [12].

VCA: Biopsies of the abdominal wall were all 4mm-punch biopsies. VCA biopsies were performed when there was an indication either for the bowel (indication-driven intestinal biopsies) or the skin (rash).

It should be noted that early in the study, we were strictly adherent to the early, intensive intestinal biopsy protocol. However, with growing experience we have become more reliant on utilizing the VCA skin as a surrogate marker [6] for intestinal rejection and therefore have significantly decreased the number of protocol biopsies.

Rejection treatment

The standard first-line treatment for intestinal and/or VCA rejection was high dose iv steroid; 500mg bolus on 3 following days. Alemtuzumab was used in cases of steroid-resistant rejection episodes (n=2).

Immunology assessment

HLA typing

All patients were typed for HLA-A, B, C, DRB1, DRB3/4/5, DQB1 and DPB1 loci using polymerase chain reaction sequence-specific primer (PCR-SSP) methods.

HLA antibody monitoring

Before transplant, 3-monthly serum samples (monthly if the patient was sensitised) were tested by solid phase bead-based Luminex assays using LABScreen antibody screening (LSM12) and identification kits (LS1PRA, LS2PRA, LS1A04, LS2A01; One Lambda Inc. Canoga Park, CA).

Post-transplant, routine HLA antibody monitoring was performed at 1, 3, 6, 9 and 12 months then annually thereafter, as well as at the time of clinical events. All DSA were identified by Single Antigen bead (SAB) assays.

All serum samples were tested undiluted and those requiring SAB testing for antibody specification were pre-treated with 0.3% ethylene-diamine-tetra-acetic acid (EDTA) [13]. Following the treatment, 10µl serum was incubated with 2.5µl of beads for 30 minutes. Following washing, bound antibody was detected by incubating the beads with 100 µL phycoerythrin-conjugated goat antihuman IgG antibody (One Lambda Inc. Canoga Park, CA) for 30 minutes in the dark. Samples were analysed using a Luminex 100 IS fluorescence detector system (Luminex Corp. Austin, TX).

Crossmatch

At the time of transplantation, all patients were crossmatched against their donors by flow cytometry and complement dependent cytotoxicity (CDC). In the CDC crossmatch, patient sera were tested with and without 1,4-Dithiothreitol to distinguish between IgG and IgM antibodies.

Data assessment

In addition to donor, recipient and surgery related characteristics, the course of DSA binding was analysed and recorded as the highest median fluorescence intensity (MFI). MFI of class I and II antibodies, as well as the cumulative MFI of both antibody classes were measured. A dnDSA was considered as positive with an MFI level >1000. The antibodies were identified and analysed for 3 different time periods: 0-6 months, 6-12 months and after 12 months.

Statistical analysis

Statistical analyses were performed with SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA). Graft survival was calculated using Kaplan-Meier estimates. Graft loss was defined as either loss of the organ or patient death with a functioning organ. Differences between survival curves were tested for significance by the log-rank test. Cumulative incidence curves were performed for occurrence of dnDSA. Values if not otherwise indicated are means ± SD.

Results

Demographics

Recipient and donor demographics and all relevant surgical factors are shown in Table 1 for patients transplanted with or without a VCA.

In the overall cohort, the median recipient age was 39.5 (range 23-73) years and 56.3% were male. The mean recipient BMI was 20.8 ± 2.9 kg/m^2. The most common cause of intestinal failure was inflammatory bowel disease (n=8), followed by visceral neuropathy (n=6). Re-transplantation of both intestine and abdominal wall was performed in 1 patient.

Twenty-four patients (24/32, 75%) received an isolated intestinal transplant. Twelve (12/24, 50%) had a concurrent VCA, including one small bowel and VCA re-transplant. Eight recipients (8/32, 25%) received a MMVTx, including stomach, duodenum, pancreas, small bowel and right hemicolon; six (6/8, 75%) transplants were combined with a VCA.

In 24 out of 32 cases, systemic venous drainage was chosen. The mean number of blood units administered per patient was 7 ± 7.7. Mean cold ischemia time (CIT) was 405 ± 93 minutes in the overall cohort.

In Table 1, HLA class I and II mismatches are shown for the overall cohort and the subgroups. In 21/32 (65.6%) recipients there were 4-6 HLA mismatches and in 11/32 (34.4%) cases 0-3 HLA mismatches.

Immunosuppression

According to our protocol, all our recipients received alemtuzumab induction followed by tacrolimus monotherapy. In the longer follow-up period, immunosuppressive treatment was adjusted due to renal dysfunction.

Prednisolone was commenced at a low dose (5mg OD) for all patients with renal impairment. In six patients (6/32, 18.8%) there was evidence of calcineurin inhibitor nephrotoxicity as demonstrated by a mean decline of 45ml/min (range 25-70ml/min) in eGFR from pre-transplant values ($p<0.001$). All six patients were switched from tacrolimus to belatacept; all had positive Epstein-Barr virus (EBV) serology at the time of the switch. Five patients (83.3%) demonstrated an immediate improvement in eGFR. One patient demonstrated a decrease in proteinuria without a significant improvement of the eGFR.

Rejection Episodes

All rejection episodes (both intestine and skin) were biopsy proven and both intestine and VCA were biopsied in all cases of rejection. Histology revealed T-cell mediated rejection in all cases. Staining for C4d was not undertaken. Overall, 8/32 (25%) patients experienced intestinal rejection episodes. Fewer patients developed intestinal rejection in the combined ITx+VCA group (3/18, 16.7%) compared to the non-VCA group (5/14, 35.7%). One of these five non-VCA group intestinal rejection episodes was graded as severe and one was moderate. In the patients receiving a VCA, 7/18 (38.9%) had visible rejection of the skin (table 1). In the combined ITx + VCA patient group there was no evidence of rejection in the intestine without skin rejection. Moderate intestinal rejection was seen in 2/3 ITx +VCA patients; 1/3 was graded as mild. So far, there has been no evidence that the presence of the VCA leads to an increase in the frequency of intestinal rejection episodes ($p = 0.23$).

All rejection episodes (intestine or VCA) were treated with 3 pulses of methylprednisolone (500mg). Alemtuzumab was used in 2 steroid resistant rejection episodes.

Graft Survival

The overall one and three year graft survival rates are 86% and 65%. For the ITx group this was 78% and 70% and for the ITx+VCA group 94% and 57%, respectively (p=0.67). The reasons for graft loss and mortality are shown in table 2.

Donor Specific Antibodies

Before transplantation, HLA antibodies were detected in 13/32 (40.6%) patients using Luminex assays. Two (2/32, 6.3%) transplants were performed in the presence of known DSA. Pre-transplant CDC crossmatches were negative for all patients and in 1/32 transplants the known DSA (HLA-Bw4 and DQ5) resulted in a positive flow cytometry crossmatch. The other DSA positive transplant was both CDC and flow cytometry crossmatch negative (DSA: HLA-DPB1*04:01).

Post-transplant samples were available for 29/32 (90.6%) transplants. There was no change in the HLA antibody status following 8/29 (27.6%) transplants. De novo HLA antibodies were detected following 21/29 (72.4%) transplants. In 14/29 (48.3%) transplants the de novo HLA antibodies were directed against the donor (dnDSA) and in the remaining 24.1% transplants the de novo antibodies were not directed against donor HLA mismatches. In the two transplants performed in the presence of known DSA, the DSA persisted post-

transplant, but there were no dnDSA. In 4/14 (28.6%) cases dnDSA developed against HLA class I, in 3/14 cases against HLA class II (21.4%) and in 7/14 (50%) cases against both HLA class I and II.

The addition of a VCA did not increase the incidence of development of dnDSA post-transplant. Six of 16 (37.5%) VCA cases developed dnDSA compared to 8/13 (61.5%) in the group without a VCA (p=0.198). Furthermore, patients who received an MMVTx were not more likely to develop dnDSA than patients receiving an SBTx. Two of six (33.3%) MMVTx recipients developed dnDSA whereas 12/23 (52.2%) SBTx recipients formed dnDSA (p=0.651).

There was no significant association between the presence of HLA sensitization pre-transplant and the development of HLA antibodies following transplant (p=0.549). Also, the number of blood units received in the peri- and post-operative period did not correlate significantly with the development of de novo HLA antibodies (p=0.632) or dnDSA (p=0.417).

In the VCA group three intestinal rejections were identified, whereas there were five confirmed intestinal rejections in the non-VCA group (p=0.730). It should be noted that 6/14 cases (42.9%) with dnDSA had at least one episode of intestinal rejection. In 4/6 (66.7%) cases dnDSA were detected before or around the occurrence of intestinal rejection. In one case (1/6, 16.7%) dnDSA appeared one year after intestinal rejection. In another case (1/6, 16.7%) dnDSA were detected after the change in immunosuppression (figure 1). Only 2/15 (13.3%) dnDSA negative cases had an episode of intestinal rejection. The switch to a non-calcineurin inhibitor was not associated with higher incidence of dnDSA (p=0.311).

De novo DSA, MFI levels and their development over time

The development of dnDSA over time was analysed; in 9/14 (64.3%) cases, dnDSA developed during the first six months; in one case (7.1%) between six and twelve months, and in 4/14 (28.6%) cases after one year. The MFI levels and the dnDSA for each patient are shown in figure 1.

0-6 months post-transplant

In the first six months after transplantation, dnDSA were detected in 9/29 (31%) patients. Four of the nine cases belonged to the VCA group and 5/9 to the non-VCA group. Four patients had class I antibodies, three class II and two had both class I and II dnDSA.

6-12 months post-transplant

Between six and twelve months after transplantation, there were five patients with dnDSA. Of the nine patients who had developed dnDSA within the first six months post-transplant, 2/9 lost their grafts. DSA became undetectable in 3/9 patients and persisted in 4/9 patients.

>12 months post-transplant

In this group, there were eight patients with dnDSA. Four were in the VCA group and four in the non-VCA group. Two patients had class I, two class II and four both classes. Four of the eight cases in the >12 months group had persistent dnDSA, whereas the remaining four developed late dnDSA. A triggering factor could be found for all late dnDSA cases: #1 a change to the immunosuppressive therapy (switch from tacrolimus to belatacept); #2 dnDSA

occurred after a Norovirus infection and in patents #3 and #4 dnDSA appeared after anti-rejection treatment and a consequent change in maintenance immunosuppression. It should be noted here that two patients with late persistent dnDSA were known to be non-adherent.

Impact of donor specific antibodies

There was no statistically significant difference in one and three-year overall graft survival stratified for the presence of DSA; 85% and 51% in the DSA positive group respectively; 93% and 85% in the DSA negative group respectively (p=0.235).

When stratified for the presence of dnDSA, the one and three-year overall graft survival was 85% and 54% respectively in the dnDSA positive group; 93% and 85% graft survival in the dnDSA negative group (p=0.286); figure 2A. In addition, we analysed cumulative graft survival in the dnDSA positive group after the appearance of antibodies. The cumulative incidence curve is shown in figure 2B.

The cumulative incidence of dnDSA after transplantation is displayed in figure 3A. The cumulative incidence curve for occurence of dnDSA after transplantation stratified for inclusion of a VCA is shown in figure 3B.

The cumulative MFI levels for class I dnDSA were significantly higher in the patients who lost their graft; 17.384 ± 13.050 than in those with a functioning graft 4.038 ± 4.302, p= 0.03. The cumulative MFI levels for class II dnDSA were also higher in the graft loss group, but this was not statistically significant; 14.500 ± 21.022 vs. 3.520 ± 2.603 respectively (p=0.38).

Discussion

This is the first analysis to explore the immunological impact of VCA in combination with solid organ transplantation. The addition of a VCA to an intestinal transplant raises the question of whether sensitization is increased by transplanting more tissue and especially skin.

Skin is regarded as the most immunogenic tissue [14, 15] and this is potentially a benefit when transplanting along with a visceral transplant. As well as using the abdominal wall to achieve effective closure of the abdominal cavity after intestinal transplantation, detection of rejection in the skin from the same donor might provide some “lead time” before rejection is apparent in the visceral organ. Furthermore, and a particular advantage in intestinal transplantation, it might also help to distinguish rejection from an infection. With longer follow up and increasing experience, we can confirm our previous findings regarding the immunological significance of the abdominal wall VCA [6, 16, 17].

Most importantly, in this patient cohort the presence of the VCA did not appear to increase sensitization or the formation of dnDSA compared to transplants performed without a VCA. De novo DSA seem to be less frequent in the VCA inclusive group, albeit not statistically significantly. A potential explanation for this could be that rejection was diagnosed and treated earlier in these patients, before it caused significant tissue injury and formation of dnDSA. Rejection of the intestine in the absence of prior or concomitant skin rejection has yet to be seen, as there has been no single case where intestinal rejection was not accompanied by a skin rejection. Overall, these

early data suggest that VCA provides a valuable immunological monitoring system and can be used as a sentinel [6, 16, 17].

Besides early dnDSA, we identified patients that developed late dnDSA (>12months). With the exception of a single case of infective gastroenteritis, all other late dnDSA coincided with either changes in immunosuppression or non-adherence. As previously reported by other studies [18], calcineurin inhibitor minimization or cessation either through non-adherence or driven by physician (intended to reverse drug nephrotoxicity) was a major risk factor for development of dnDSA with subsequent allograft rejection and loss [19]. Changes in immunosuppression were found to trigger immunological events in our cohort, but this was not statistically significant.

This study also evaluated the impact of dnDSA on allograft survival. The one and three-year graft survival was inferior in the group of patients who developed dnDSA. The difference was not significant which could be a result of the small number of 14 dnDSA positive patients in our cohort. This is consistent with previous studies regarding the role of DSA. The Miami group [20], who first described abdominal wall transplantation in visceral transplant patients, were also the first group to study the impact of DSA on intestinal allograft rejection. In their cohort of 15 grafts in 13 patients, clinical rejection episodes were significantly associated with the presence of DSA; reduction of MFI levels was associated with clinical resolution of the rejection. Recently, the UCLA group [21] has reported in a cohort of 109 intestinal transplants, an association between DSA and accelerated intestinal allograft failure. They showed that dnDSA against class II HLA antigens were persistent, which is consistent with our observation of persistent class II dnDSA.

A recent publication demonstrated that blood transfusion was an independent predictive factor for the development of dnDSA [22] but, although antibody-mediated rejection was more likely in transfused patients, blood transfusion was not a significant independent factor. In contrast to these findings, our study did not show a correlation between total blood units transfusion and intestinal rejection episodes (p=0.756) or dnDSA development (p=0.417). However, further investigations are needed on the effect of blood transfusions early after ITx and VCA.

The incidence of dnDSAs has been found to be 48.3% in our cohort and is therefore higher when compared with data from other centres (18-40%) [8, 9, 20, 23]. There are probably two main reasons to explain this higher incidence. Firstly, we utilize alemtuzumab as induction agent for all our transplants. Alemtuzumab induction has been associated with reduced acute rejection episodes as shown by the 3C study in renal transplantation [24]. However, on the other hand, it has been associated with a higher incidence of dnDSAs compared to Basiliximab or ATG [25]. Secondly and more importantly, our cohort does not include any liver grafts and we therefore lose the favourable liver effect [1, 26].

There are some limitations to this study, as it is retrospective and single-centre. However, this is a unique topic given that there are not currently any other centres worldwide routinely performing the combination of ITx+VCA. Sample size is therefore limited and collaborations are yet to be sought.

In conclusion, the development of DSA in intestinal transplantation, as in other organ types, is detrimental to the long-term survival of the graft. Our data so

far suggest that combining an abdominal wall VCA with an intestinal transplant does not increase the incidence of dnDSA.

Figure and Table Legends

Figure 1. Timeline showing the development of dnDSA in the two patient cohorts (ITx and ITx+VCA), MFI levels and association with immunological events.

Figure 2A. Kaplan Meier survival plot for intestinal allografts stratified by the presence of dnDSA (p=0.286).

Figure 2B. Cumulative graft survival plot for survival post dnDSA occurrence.

Figure 3A. Cumulative incidence curve for dnDSA development after intestinal transplantation

Figure 3B. Cumulative incidence curve for dnDSA stratified for inclusion of VCA after intestinal transplantation.

Table 1. Demographics of intestinal and modified multivisceral transplants, 2008 - 2015 (n=32)

Table 2. Reasons for graft loss and death after combined intestinal and abdominal wall transplant

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Table 1: Demographics of intestinal and modified multivisceral transplantations between 2008 and 2015 (n=32)

Characteristics	Overall Cohort (n=32)	VCA (n=18)	no VCA (n=14)	dnDSA+ (n=14)	dnDSA- (n=15*)
Recipient age in years (median (min-max))	39.5 (23-73)	37.5 (26-69)	42.5 (23-73)	35 (23-47)	44 (25-69)
Recipient BMI kg/m2 (mean, SD)	20.8 ± 2.9	20.7 ± 2.7	21 ± 3.2	21.2 ± 3.6	20.3 ± 1.9
Recipient male gender (n, %)	18 (56.3%)	11 (61.1%)	7 (50%)	9 (64%)	8 (53%)
Prior Tx (n, %)	1 (3.1%)	1 (5.6%)	0	1/14 (7%)	0
Donor age in years (median (min-max))	24.5 (8-51)	22.5 (8-49)	31.5 (10-51)	30.1 (10-51)	27 (8-46)
Donor BMI in kg/m2 (mean, SD)	22.1 ± 2.2	22.1 ± 2.5	22.1 ± 2	22.2 ± 1.5	22 ± 2.8
Donor male gender (n, %)	17 (53%)	9 (50%)	8 (57.1%)	8 (57%)	7 (47%)
Cause of small bowel syndrom/failure (n, %)					
Inflammatory bowel disease (IBD)	8 (25%)	5 (27.8%)	3 (21.4%)	7 (50%)	1 (6.7%)
Enteritis/enterocolitis other than IBD	4 (12.5%)	3 (16.7%)	1 (7.1%)	1 (7.1%)	3 (20%)
Pseudomyxoma peritonei (PMP)	4 (12.5%)	4 (22.2%)	0	0	2 (13.4%)
Neoplasia other than PMP	5 (15.6%)	3 (16.7%)	2 (14.3%)	1 (7.1%)	4 (26.6%)
Mesenteric thrombosis	5 (15.6%)	1 (5.6%)	4 (28.6%)	3 (21.5%)	1 (6.7%)
Visceral neuropathy	6 (18.8%)	2 (11.1%)	4 (28.6%)	2 (14.3%)	4 (26.6%)
Isolated Intestinal Tx (n, %)	24 (75%)	na	12 (85.7%)	12 (85.7%)	11 (73.3%)
- Intestinal Tx with VCA (n, %)	12/24 (50%)	12 (66.7%)	na	5/12 (41.7%)	7/11 (63.6%)
Modified Multivisceral Tx (n, %)	8 (25%)	na	2 (14.3%)	2 (14.3%)	4 (26.7%)
- Modified Multivisceral Tx with VCA (n, %)	6/8 (75%)	6 (33.3%)	na	1/2 (50%)	3/4 (75%)
Systemic vs Portal Venous Drainage (n, %)	24 (75%)	12 (66.7%)	12 (85.7%)	12 (85.7%)	11 (73.3%)
Cold ischemia time in hours (mean, SD)	6.8 ± 1.5	6.6 ± 1.6	7 ± 1.5	6.5 ± 1.2	6.7 ± 1.6

Class I mm					
HLA A mm (mean, SD)	1.16 ± 0.68	1.11 ± 0.76	1.21 ± 0.56	1.25 ± 0.68	1.07 ± 0.73
- 0 and 1 (n, %)	22 (68.8%)	12 (66.7%)	10 (71.4%)	9 (64.3%)	11 (73.3%)
- 2 (n, %)	10 (31.2%)	6 (33.3%)	4 (28.6%)	5 (35.7%)	4 (26.7%)
HLA B mm (mean, SD)	1.41 ± 0.76	1.56 ± 0.62	1.21 ± 0.89	1.63 ± 0.62	1.07 ± 0.83
- 0 and 1 (n, %)	14 (43.8%)	7 (38.9%)	7 (50%)	4 (28.6%)	9 (60%)
- 2 (n, %)	18 (56.2%)	11 (61.1%)	7 (50%)	10 (71.4%)	6 (40%)
Class II mm					
HLA DR mm (mean, SD)	1.31 ± 0.74	1.28 ± 0.75	1.36 ± 0.75	1.44 ± 0.73	1.14 ± 0.77
- 0 and 1 (n, %)	17 (53.1%)	10 (55.6%)	7 (50%)	7 (50%)	9 (60%)
- 2 (n, %)	15 (46.9%)	8 (44.4%)	7 (50%)	7 (50%)	6 (40%)
Total number of mm (median (min-max))	4 (0-6)	4 (1-6)	4 (0-6)	5 (1-6)	3 (0-6)
Total number of blood units (mean, SD, median, IQR)	7 ± 7.7 4, 7	8.9 ± 9.2 4, 8	4.4 ± 3.9 3, 6	4.9 ± 2.9 5, 5	6.6 ± 7.4 3, 11
Pre-existing DSA (n, %)	2 (6.3%)	2/16* (11.8%)	0	0	2 (13.4%)
De novo DSA (n, %)		6/16* (35.3%)	8/13* (61.5%)	Na	0
Acute rejection intestinal Tx (n, %)	8/32 (25%)	3 (16.7%)	5 (35.7%)	6/14 (42.9%)	2/15 (13.3%)
Acute rejection VCA (n, %)	7/18 (38.9%)	7 (38.9%)	na	2/6 (33.3%)	5/10 (50%)

dnDSA = de novo DSA
na = not applicable in this group
* no follow up for dnDSA in 3 cases

Table 2: Reasons for graft loss and death after combined intestinal and abdominal wall transplant

Characteristics	Intestinal Tx	Intestinal Tx+VCA
Graft loss (n=10)		
Pancytopenia/Sepsis	1	0
Rejection	2	2
MOF/Liver ischemia	1	0
Mesenteric thrombosis	1	0
Venous thrombosis	1	0
Duodenal leak	0	1
CMV enteritis	0	1
	6	4
Death (n=12)		
Sepsis	2	1
Pulmonary embolism	1	0
MOF/Liver ischemia	1	0
Brain abscess	1	0
Mesenteric thrombosis	1	0
CMV enteritis	0	1
Cerebral oedema/Hyperammonemia	0	1
GVHD	0	1
Tx pancreatitis	0	1
Upper GI bleed	0	1
	6	6

Figure 1

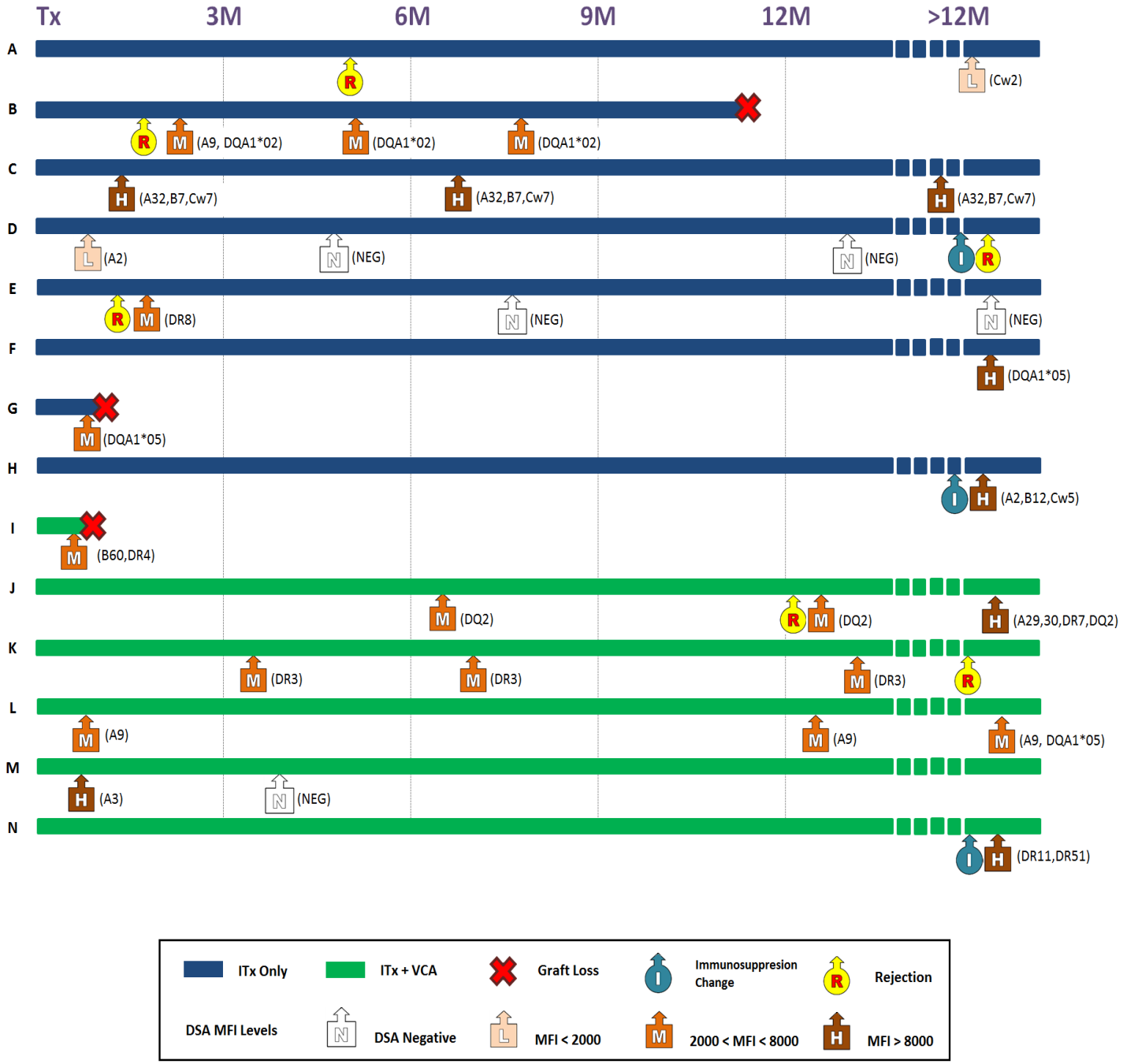
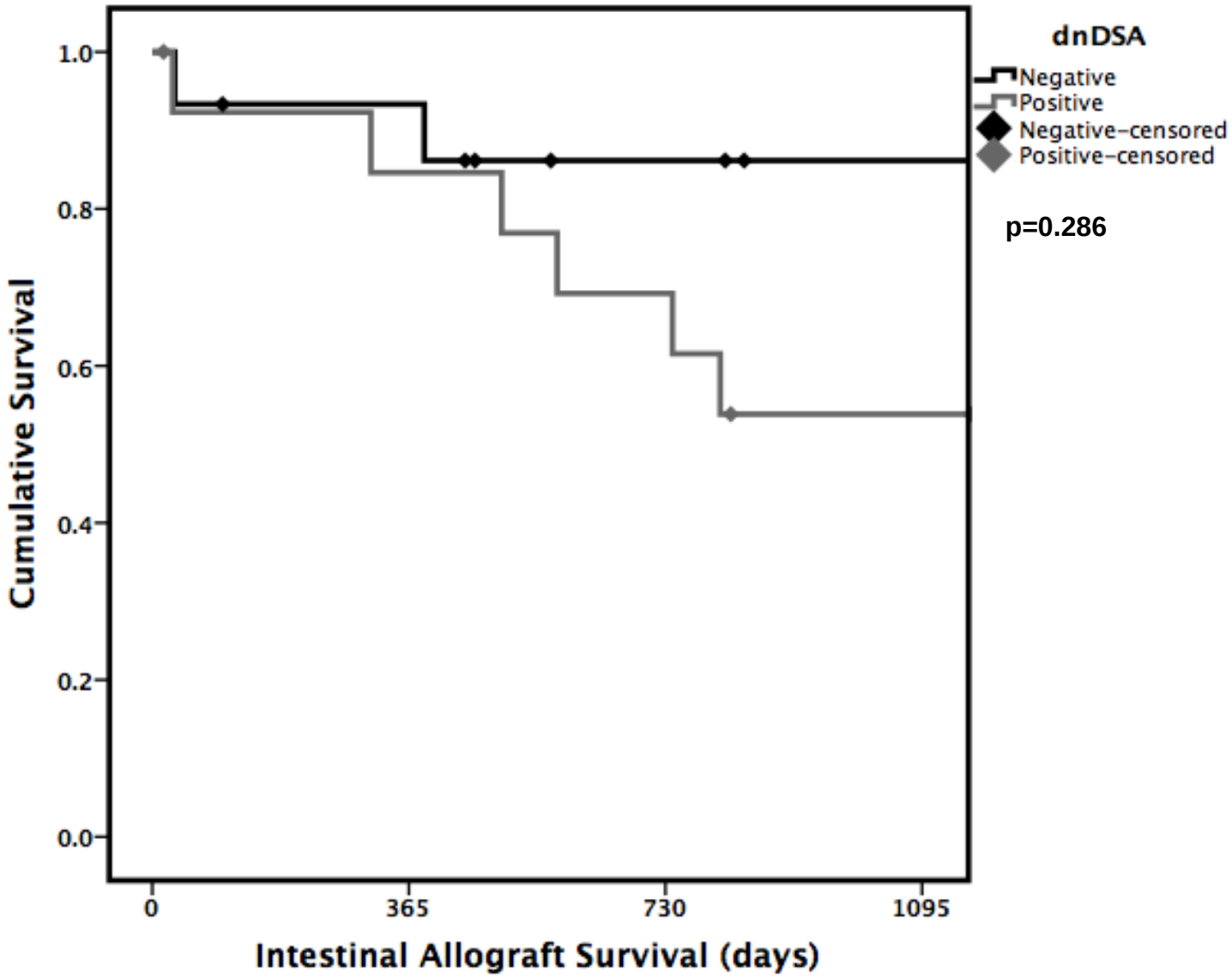
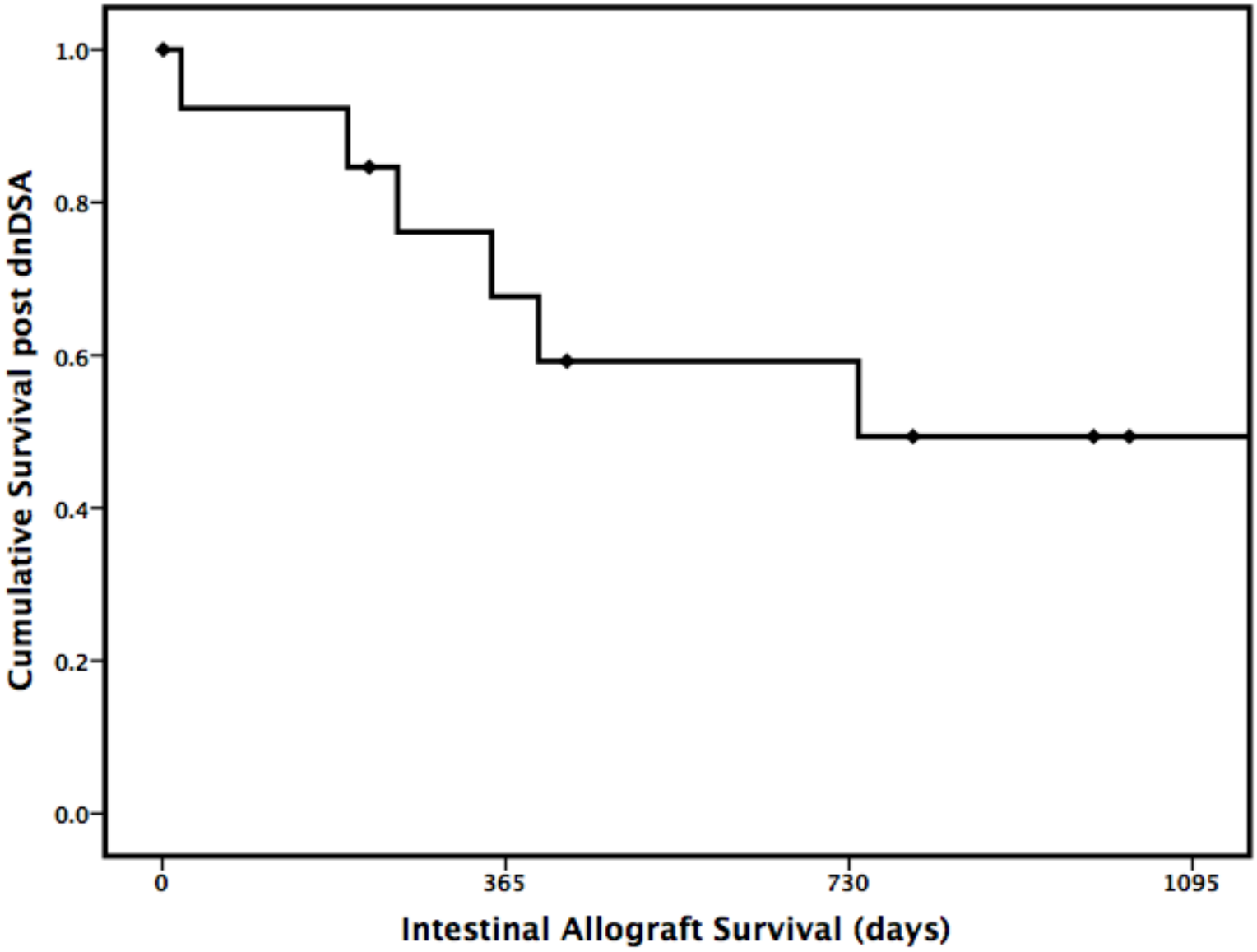

Tx
3M
6M
9M
12M
>12M
A
B
C
D
E
F
G
H
I
J
K
L
M
N
(Cw2)
(A9, DQA1*02)
(DQA1*02)
(DQA1*02)
(A32,B7,Cw7)
(A32,B7,Cw7)
(A32,B7,Cw7)
(A2)
(NEG)
(NEG)
(DR8)
(NEG)
(NEG)
(DQA1*05)
(DQA1*05)
(A2,B12,Cw5)
(B60,DR4)
(DQ2)
(DQ2)
(A29,30,DR7,DQ2)
(DR3)
(DR3)
(DR3)
(A9)
(A9)
(A9, DQA1*05)
(A3)
(NEG)
(DR11,DR51)
ITx Only
ITx + VCA
Graft Loss
Immunosuppresion Change
Rejection
DSA MFI Levels
DSA Negative
MFI < 2000
2000 < MFI < 8000
MFI > 8000

Figure 2A



	Total	1 yr	2 yr	3 yr
Negative dnDSA	15	13	9	7
Positive dnDSA	14	11	9	6

Figure 2B



	Total	1 yr	2 yr	3 yr
Number at risk	14	8	6	2

Figure 3A

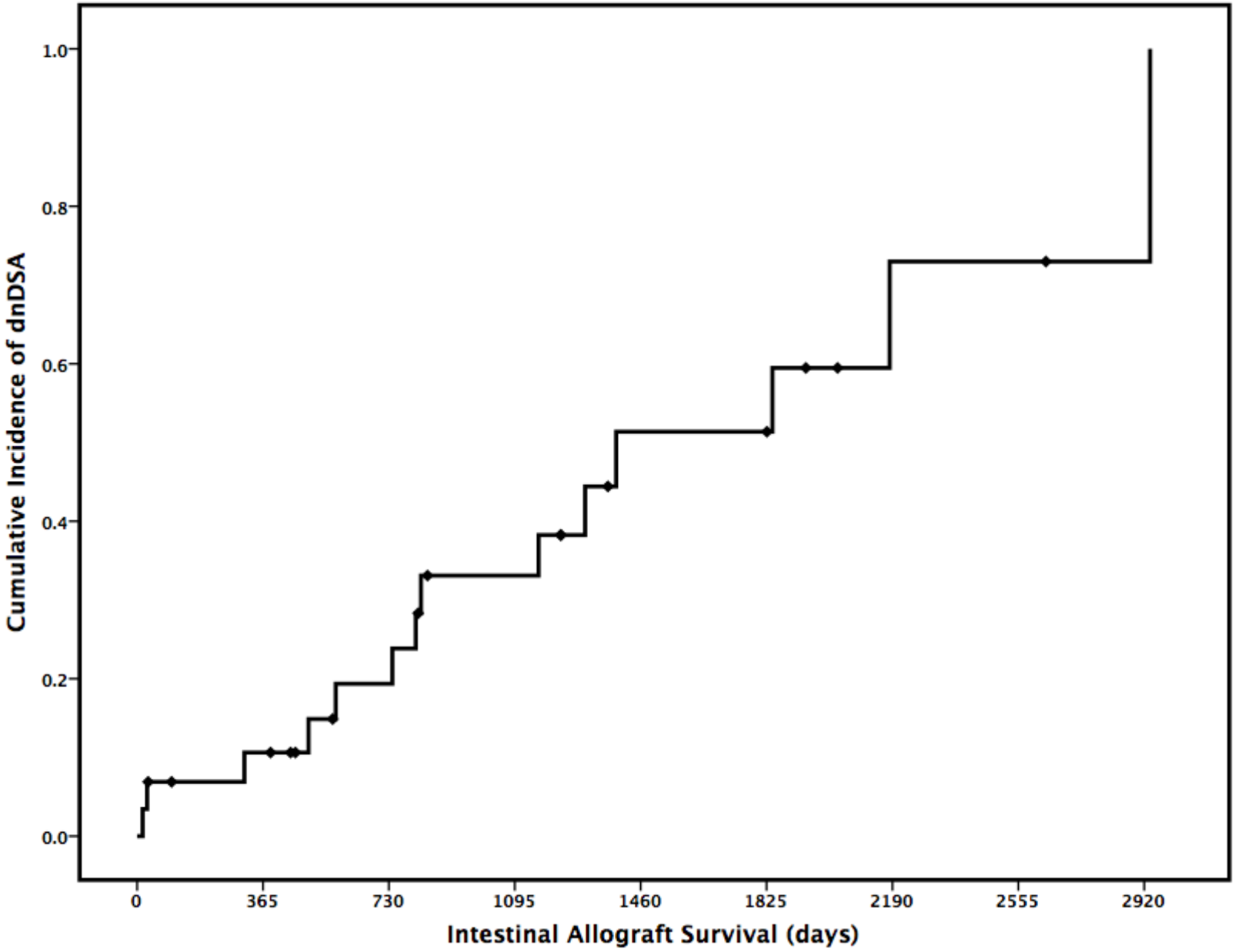
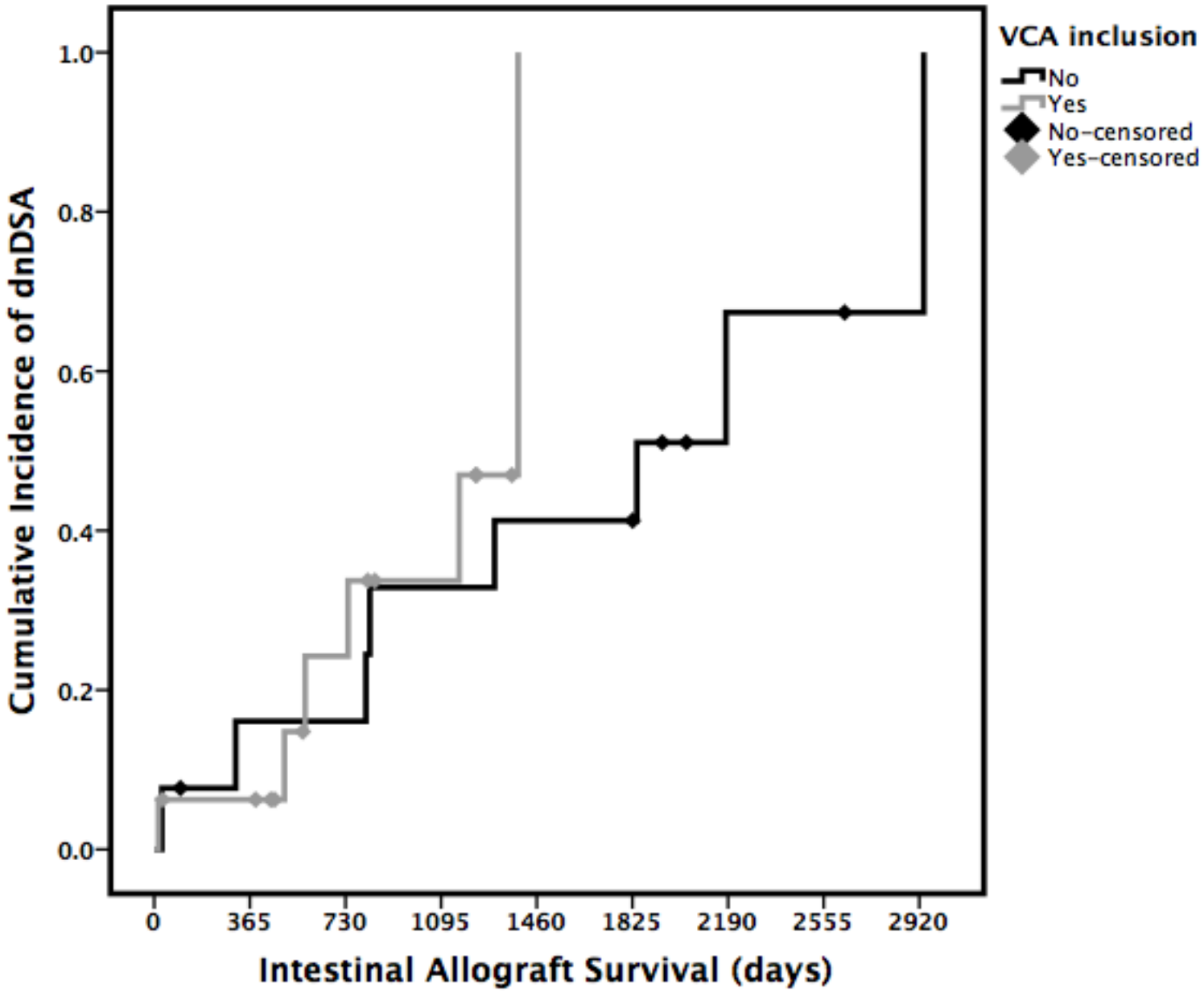

Cumulative Incidence of dnDSA
1.0
0.8
0.6
0.4
0.2
0.0
0
365
730
1095
1460
1825
2190
2555
2920
Intestinal Allograft Survival (days)

Figure 3B


VCA inclusion
No
Yes
No-censored
Yes-censored
Cumulative Incidence of dnDSA
1.0
0.8
0.6
0.4
0.2
0.0
0
365
730
1095
1460
1825
2190
2555
2920
Intestinal Allograft Survival (days)