Article type : Original Article

Steroid Free Three Drug Maintenance Regimen for Pancreas Transplant Alone: Comparison of Induction with Rabbit Antithymocyte Globulin +/- Rituximab

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Abbreviations: cytomegalovirus (CMV), donor specific antibody (DSA), glomerular filtration rate (GFR), mycophenolate mofetil (MMF), pancreas transplant alone (PTA), rabbit antithymocyte globulin (rATG), United Network for Organ Sharing (UNOS)

This is the author's manuscript of the article published in final edited form as:

Fridell, J., Mangus, R., Chen, J., Taber, T., Cabrales, A. E., Sharfuddin, A., … Powelson, J. (n.d.). Steroid Free Three Drug Maintenance Regimen for Pancreas Transplant Alone: Comparison of Induction with Rabbit Antithymocyte Globulin +/- Rituximab. American Journal of Transplantation, 0(ja). https://doi.org/10.1111/ajt.14921

Graft survival following pancreas transplant alone (PTA) is inferior to other pancreas transplants. Steroid elimination is appealing, but a two drug maintenance strategy may be inadequate. Additionally, recipients tend to have diabetic nephropathy and do not tolerate nephrotoxic medications. A three-drug maintenance strategy permits immunosuppression through different mechanisms as well as an opportunity to use lower doses of the individual medications. Induction consisted of five doses of rabbit antithymocyte globulin (1 mg/kg/dose). As of October 2007, a single dose of rituximab (150 mg/m2) was added. Maintenance consisted of tacrolimus, sirolimus and mycophenolate mofetil. From 2004 to 2017, 166 PTA were performed. Graft loss at 7- and 90- days were 4% and 5%, and one year patient and graft survival were 97% and 91%. Comparing induction without and with rituximab, there was no significant difference in 7 or 90 day graft loss, 1 year patient or graft survival or in the rate of rejection or infection. Rabbit antithymocyte globulin induction and steroid withdrawal followed by a three drug immunosuppression regimen is an excellent strategy for PTA recipients.

Introduction: Pancreas transplantation has the potential to render candidates with Type 1 diabetes euglycemic without the requirement for administration of exogenous insulin. This procedure requires a major abdominal operation and a lifelong commitment to immunosuppression, so it is currently exclusively offered as a treatment option to diabetics that require another extrapancreatic transplant, usually a kidney transplant for end stage diabetic nephropathy, or for patients with potentially life threatening complications of diabetes such as hypoglycemia unawareness. Historically, graft survival following pancreas transplant alone (PTA) has been inferior to that of other pancreas transplants due to a higher incidence of chronic rejection and late allograft failure[1]. This may be related to the fact that candidates for PTA tend to be younger than those presenting for a kidney and a pancreas. We

have previously reviewed the impact of age on pancreas transplant outcomes and have demonstrated inferior outcomes in the youngest population which we attributed to a more virulent immune system or to a greater frequency of noncompliance in the younger recipients[2]. Alternatively, the development of renal failure may also have an important impact on the immune system making chronic rejection less likely in recipients requiring both organs.

Steroid elimination is very appealing in the setting of pancreas transplantation because of their extensive side-effect profile and poor tolerability [3-6]. In particular, for pancreas transplantation, concerns about insulin resistance and development of type II diabetes mellitus related to steroid therapy have been raised. This was one of the essential components of the Edmonton immunosuppression protocol for Islet transplantation[7] and has been well described in pancreas transplantation [8-10]. That being said, a two-drug maintenance strategy may be inadequate for PTA. Additionally, although not enough to qualify for kidney transplantation, recipients tend to have some degree of diabetic nephropathy and do not tolerate nephrotoxic medications at full dose. This patient population tends to also consist of particularly brittle diabetics where gastroparesis and other diabetes related bowel motility disorders may be quite common. For this reason, full dose mycophenolate mofetil or mycophenolic acid may not be tolerated. A three-drug maintenance strategy permits the simultaneous increase in overall immunosuppression while using lower doses of the individual medications in order to avoid toxicity.

There have been several reports associating development of donor specific antibody (DSA) with late allograft failure[11, 12]. We have previously reported our liver transplant immunosuppression protocol that included delayed rabbit antithymocyte globulin (rATG) (48 hour delay, three doses for total of 6 mg/kg) and a single dose of rituximab (1.5 mg/m^2)[13]. Based on this protocol rituximab was added for all PTA as well in an attempt to decrease

development of DSA and hopefully decrease longterm allograft loss to chronic rejection. We have previously reported the impact this has had on development of DSA in this patient population.[14]

This study is a retrospective single center analysis that describes the results using a steroid free rATG induction protocol with three drug immunosuppression maintenance therapy and comparing prior to and following introduction of rituximab as a component of induction.

Materials and Methods: The medical records for all adult, deceased donor PTA transplants performed at Indiana University between January 2004 and September2017 were reviewed (n=166). Data were extracted from the comprehensive transplant recipient registry maintained at our center, individual written and electronic medical records, and the original donor medical history. Inclusion criteria for this analysis included all PTA recipients. Pancreas retransplants, even if performed early, were included in this analysis.

All recipients were listed for transplantation at Indiana University according to standard procedures and protocols as established by our own center and the United Network for Organ Sharing (UNOS). During the study period, in order to qualify for pancreas transplant listing at our institution, the potential recipient had to be insulin dependent with a fasting serum C-peptide level < 2 ng/ml. For PTA, the recipients had to demonstrate preserved renal function, usually with a creatinine clearance of at least 50 ml/min/1.73m^2.

Pancreas allografts were typically procured using an en-bloc technique following aortic flush with preservation solution and topical cooling with saline slush as previously described [15, 16]. The recipient operation was performed through a midline incision. The pancreas was routinely positioned with the tail toward the pelvis and the head and duodenum

oriented superiorly in order to facilitate the enteric anastomosis. Systemic venous drainage was performed to the vena cava or to the right common iliac vein. Arterial perfusion of the allograft was routinely established from the right common iliac artery, although on rare occasions where this vessel was found to be diseased or had been the site for arterial anastomosis for a prior transplant, the inflow would be established either from the aorta or the left common iliac artery. All pancreas allografts were drained enterically using a stapled technique as described elsewhere [17].

The induction immunosuppression protocol consisted of five doses of rATG (1 mg/kg/dose) and maintenance (initiated post-operative day 1)with tacrolimus (target trough 6-8 ng/ml), sirolimus (target trough 3-6 ng/ml) and mycophenolate mofetil (MMF) (500 mg po bid)[8, 18]. Steroids were exclusively used as a premedication for rATG and were discontinued following induction in all recipients. As of October 2007, due to the higher incidence of chronic immunologic graft loss in the PTA population, we have also added a single dose of rituximab (150 mg/m^2) as well on post-operative day #1. In certain situations where the side effects of the maintenance immunosuppression were not well tolerated, MMF was replaced with either mycophenolic acid or azathioprine. In some instances where only two of the three maintenance immunosuppression medications were tolerated, a monthly infusion of basiliximab was added to the maintenance immunosuppression regimen. All recipients received routine perioperative antibiotics, prophylaxis against cytomegalovirus (CMV) with oral valgancyclovir and prophylaxis against Pneumocystis jiroveci pneumonia with trimethoprim and sulfamethoxazole, unless contraindicated. Systemic anticoagulation was not routinely used unless the patient had a specific history of a coagulation disorder.

Primary transplant outcomes included 7-day and 90-day pancreas allograft loss and 1-year pancreas allograft and patient survival. Pancreas allograft failure was defined by dependence on subcutaneous insulin administration. All occurrences and causes of graft loss

or patient death were included in the final analysis. Demographic data were compared using standard chi-square and analysis of variance (ANOVA) testing. Retrospective review of data from the transplant center database was approved by the institutional review board of the Indiana University School of Medicine.

Results: From 2004 to 2017, 166 PTA were performed. Donor and recipient demographics are presented in Table 1. The median follow-up for the entire population was 80 months. 6% of cases were retransplants. 31% of donor recipient combinations were CMV positive to negative (high risk). A prior diagnosis of gastroparesis was noted for 20% of the recipients. Outcomes for the entire PTA patient population are presented in Table 2. Median length of stay was 7 days and readmissions within the first 3 months was 49% of which 29% were related to dysmotility and dehydration. Specifically, graft loss at 7 and 90 days were 4% and 5% respectively and one-year patient and pancreas allograft survival were 97% and 91% respectively. The majority of patients tolerated a three drug maintenance regimen with calcineurin inhibitor, sirolimus and either mycophenolate mofetil, mcophenolic acid or azathioprine with only 25 patients (15%) requiring monthly infusion of basiliximab as a third agent. Within the first year, the Incidence of infection was 43% (CMV 11%). There were 31 patients treated for rejection (19%), all of which were treated with bolus steroids and with 22 also receiving rATG treatment. 11 patients were treated more than once for rejection. Five-year pancreas allograft survival was 68%. Median fasting c-peptide was 2.0 ng/ml with a median HBA1C of 5.5%. In terms of renal function, the median glomerular filtration rate (GFR) prior to transplant was 90 ml/min/1.73m^2 with a range from 97 to 42 ml/min/1.73m^2. However, many of patients were reported as GFR>90 ml/min/1.73m^2, so we do not know precisely what the upper limit is. There were only 3 patients with GFR < 50 ml/min/1.73m^2 and 17 patients with GFR < 60 ml/min/1.73m^2. At one year post-transplant, the median GFR had decreased to 79 ml/min/1.73m^2. In terms of body weight, this remained reasonably stable

with a change from baseline of -2.8 KG. Overall, throughout the entire study period, there were 17 deaths: Three were related to postoperative complications (1 leak, 2 aspiration pneumoniae), one from graft versus host disease, one suicide, two accidental deaths (motor vehicle accident and air embolus), two from malignancy (acute myelogenous leukemia and metastic squamous cell lung cancer), three cardiac deaths, two late deaths (renal failure with septic shock and gastrointestinal hemorrhage) and three deaths of unknown cause. Fourteen of these patients died with functioning pancreas allografts.

Comparing induction without (35 (21%), median follow-up 141 months) to with (131 (79%), median follow-up 62 months) rituximab, there was no significant difference in recipient demographics, although there were significantly more female donors (p=0.04) in the rituximab group (Table 1). Median length of stay was similar at 7 days for both groups. Readmissions within the first 3 months were similar (50% and 49%, for with and without rituximab, respectively) with 26% and 40% being related to dysmotility and dehydraton. There was no significant difference in 7-day (3% vs 6%) or 90-day (5% vs 6%) graft loss, 1 year patient (97% in both) or graft (92% vs 86%, p=0.22) survival. Median HBA1C were similar (5.5% vs 5.4%) as was median fasting c-peptide levels (2.0 ng/ml in both). The median GFR at baseline was 90 ml/min/1.73m^2 in both groups and decreased to 76 and 84 ml/min/1.73m^2 at one year. In terms of body weight, this remained reasonably stable and was similar between the groups (-1.3 kg and -3.9 kg). There was also no difference in first year rate of rejection (7% vs 6%, p=1) or infection (41% vs 51%, p=0.24) (Table 2). First year CMV rates were also comparable at 12%vs 9%, (p=0.65) with more recipients in the high risk donor positive to recipient negative in the rituxan group (33% vs 26%). ten-year allograft and patient survival comparing with and without rituximab are shown in Figures 1 and 2. The differences were not significant (p=0.65 , 0.19). As the majority of deaths were with a

functioning pancreas allograft (14/17), we have also included death censored pancreas allograft survival which did not reach significance but did demonstrate further splaying of the survival curves favoring the group that received ritximab (p=0.17).

Discussion:

Of all the different varieties of pancreas transplantation, PTA remains the most problematic for several reasons. First, the allograft survival is inferior to all other combinations of pancreas transplantation, including pancreas after kidney transplantation which is also an isolated pancreas allograft. This inferior graft survival is the result of increased early graft loss from technical issues, mostly allograft thrombosis, and from a high rate of late attrition from chronic rejection. Second, the patient population is complicated. Typically, patients present at a younger age compared to recipients that also require a renal transplant. They tend to be more brittle and have a higher incidence of gastrointestinal issues such as gastroparesis and diabetic bowel motility disorders. Finally, although their kidney function has not deteriorated enough to mandate renal transplantation, these patients' kidneys have been exposed to years of diabetes and the function is typically somewhat impaired. Although less common, this patient population also includes patients that have previously undergone total pancreatectomy for non-malignant diseases, which can represent a very difficult reoperative pancreas transplantation. With all of these factors combined, although the operation sounds straightforward, this is a physiologically and immunologically complicated recipient population.

The strategy for immunosuppression described here was developed in order to provide increased immunosuppression by combining three non-steroid agents in order to decrease pancreas allograft attrition to chronic rejection and to minimize individual medication dosing in order to decrease toxicity. Similar approaches applying the same philosophy (although

including corticosteroids) have been independently reported[19]. In terms of efficacy, the pancreas allograft short and long term survival is superior to that reported from the Organ Procurement and Transplantation Network/ Scientific Registry of Transplant Recipients (OPTN/SRTR) with 1 year survival of 74.5% and 5 year survival at 50.6%[1] and that of the International Pancreas Transplant Registry (83% 1 year and 65.5% 3 year survival for transplants performed between 2010-14)[20].

The secondary goal of this immunosuppression approach is to use the three maintenance immunosuppression medications in lower dosages in order to minimize toxicity. Tacrolimus and sirolimus are both associated with nephrotoxicity. This is particularly problematic in this patient population because, as mentioned above, although the renal function is not impaired enough to qualify for a renal allograft, the pancreas transplant alone patient population frequently present with some degree of renal impairment from the years of diabetes prior to transplantation. This regimen permits some reduction in dosage and target trough levels in order to minimize post-transplant renal impairment. Additionally, gastroparesis and diabetes related bowel dysmotility tends to be more common and more severe in this particular recipient population. Mycophenolate mofetil is potentially poorly tolerated in this circumstance given its gastrointestinal toxicity which can cause nausea, vomiting or diarrhea. This regimen permits lower dosage, as we would usually use twice the dosage in combination with tacrolimus alone. This is but one aspect of a post-transplant gastrointestinal protocol that we have applied for all of our pancreas transplant recipients which includes early introduction of oral intake, narcotic minimization with transversus abdominus plane catheters or injection in this plane with liposomal bupivacaine, routine administration of scheduled intravenous metoclopramide and subcutaneous methylnaltrexone. Despite all of these interventions, readmission most commonly occur for dysmotility and dehydration.

In terms of the contribution of rituximab to outcome, it appears that the impact was minimal if any. Episodes of acute rejection and short and longterm patient and allograft survivals were similar. We have, however, previously published a study reviewing development of donor specific antibody (DSA) after pancreas transplantation in which we demonstrated that 26% (9/35) of recipients analyzed developed DSA, but none of the 13 PTA recipients on this regimen developed DSA[14]. This was achieved without an increase in infection rate, particularly without increasing the rate of CMV infection. This may prove to be a safe and potentially effective approach, but a larger series would be required to demonstrate a significant improvement in allograft survival.

Conclusion: Rabbit antithymocyte globulin induction and steroid withdrawal followed by a steroid-free three drug immunosuppression regimen is an excellent strategy for pancreas transplant alone recipients. Rituximab as a component of induction was well tolerated, though a larger patient population with longer follow-up and specific monitoring for development of donor specific antibodies and autoantibodies would be required to determine the full impact.

Acknowledgments: This work was presented at the International Pancreas and Islet Transplantation Association (IPITA-IXA-CTS Joint Congress) meeting in Melbourne, Australia in 2015.

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

Table 1. Recipient and donor demographic data for 166 consecutive adult pancreas transplant alone patients receiving rabbit antithymocyte globulin-based induction therapy (rATG) at Indiana University from 2004 to 2017.

		Immunosuppression induction protocol		
	ALL PTA	Antithymocyte globulin	Antithymocyte globulin + Rituximab	p-value
TOTAL	**166**	**35 (21%)**	**131 (79%)**	
RECIPIENT -				
Age (years, median (SD))	40 (11)	40 (11)	40 (11)	0.76
Gender male	46%	51%	45%	0.50
Race White	92%	91%	92%	0.59
Body mass index (median (SD))	26.6 (4.3)	24.9 (4.5)	26.8 (4.2)	0.38
Weight at transplant (kg, median (SD)	74.8 (15)	69.4 (16)	75.6 (15)	0.18
Retransplant	6%	9%	5%	0.11
Follow-up months (median (SD))	80 (45)	141 (32)	62 (35)	<0.001
CMV: positive to negative	31%	26%	33%	0.15
Gastroparesis	20%	20%	20%	0.98
DONOR -				
Age (years, median (SD))	23 (10)	24 (10)	23 (11)	0.79
Gender (male)	65%	80%	61%	0.04
Race White	73%	80%	71%	0.11
Body mass index (median (SD))	24.1 (5.4)	23.7 (4.2)	25.0 (5.8)	0.27

Table 2. Pancreas transplant clinical outcomes for 166 consecutive adult pancreas transplant alone (PTA) patients receiving rATG-based induction immunosuppression at Indiana University between 2004 and 2017.

		Immunosuppression induction protocol		
	All PTA	**Antithymocyte globulin**	**Antithymocyte globulin + Rituximab**	**p-value**
Number	**166**	**35 (21%)**	**131 (79%)**	
Graft loss within 7 days	6 (4%)	2 (6%)	4 (3%)	0.37*
Graft loss within 90 days	8 (5%)	2 (6%)	6 (5%)	0.78
Length of hospital stay (days, median (SD))	7	7	7	0.99
3-month readmission (any)	49%	49%	50%	0.91
For dismotility / dehydration	29%	40%	26%	0.29
1-year post transplant				
Graft survival	91%	86%	92%	0.22
Patient survival	97%	97%	97%	0.72*
Rejection (any)	7%	6%	7%	1.00*
Infection (any)	43%	51%	41%	0.24
Infections				
Cytomegalovirus	11%	9%	12%	0.65
Bacterial (any)	36%	44%	33%	0.24
Fungal	4%	0%	5%	0.34*
Clostridium difficile	9%	12%	9%	0.55
BK virus	2%	0%	2%	1.00*
Graft function				
HbA1c (median (SD))	5.5 (0.6)	5.4 (0.6)	5.5 (0.9)	0.15
C-peptide (median (SD))	2.0 (1.6)	2.0 (1.7)	2.0 (1.6)	0.61
Patient weight change from baseline (kg)	-2.8 (12.0)	-3.9 (9.6)	-1.3 (12.6)	0.87
Glomerular filtration rate				
Baseline (median (SD))	90 (12)	90 (12)	90 (12)	0.96
1-year post transplant (median (SD))	79 (34)	84 (37)	76 (33)	0.07

* By Fisher's Exact test

Figure Legends:

Figure 1a. Cox regression pancreas transplant allograft survival following pancreas transplant alone comparing standard immunosuppression induction with and without rituximab. Figure 1b. Cox regression pancreas transplant allograft death censored survival following pancreas transplant alone comparing standard immunosuppression induction with and without rituximab.

Figure 2. Cox regression pancreas transplant patient survival following pancreas transplant alone comparing standard immunosuppression induction with and without rituximab.

Figure 1a

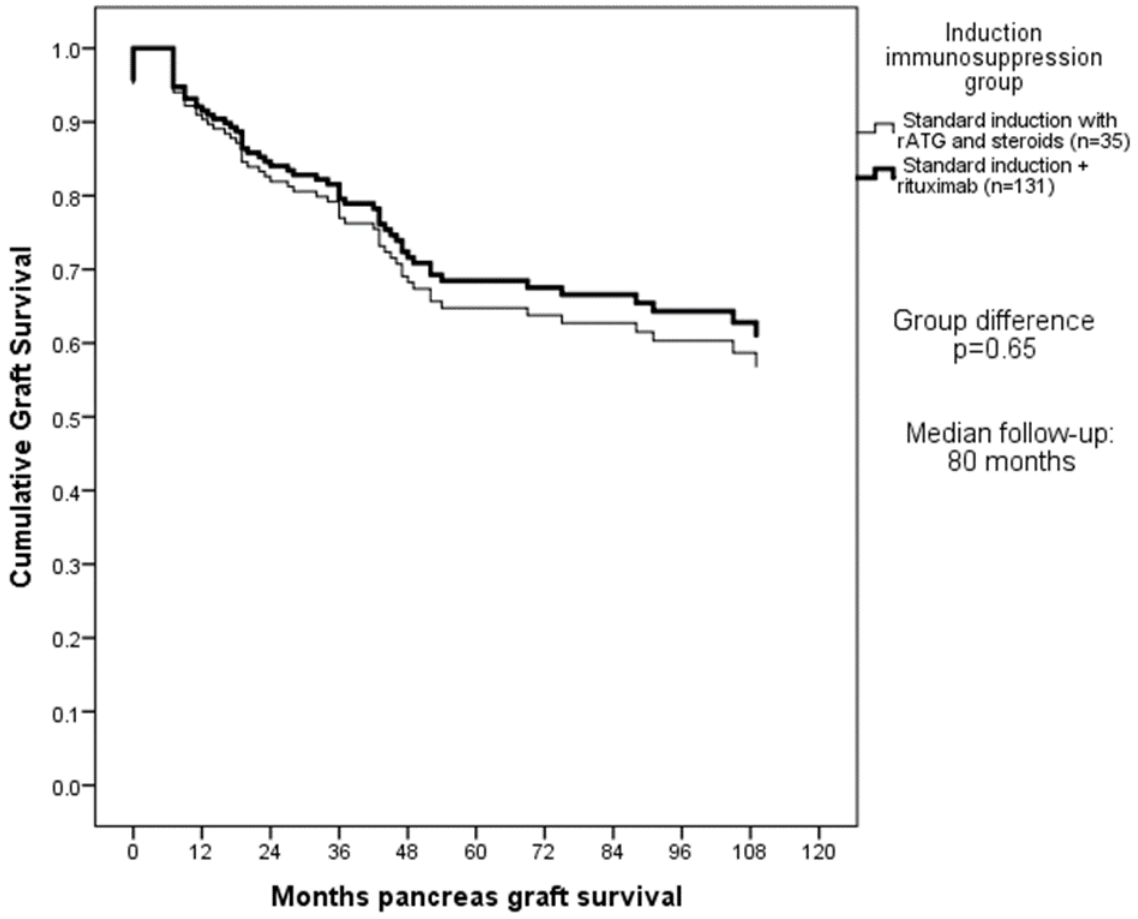


Figure 1b

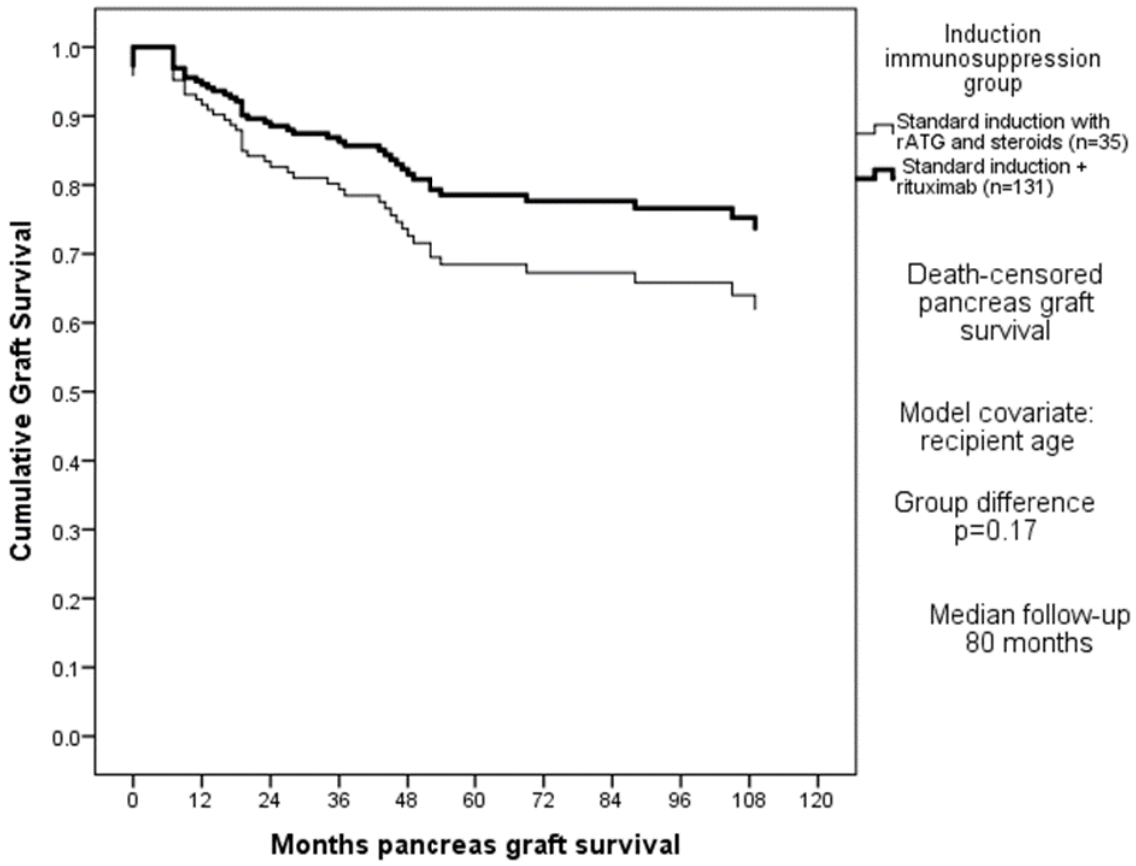
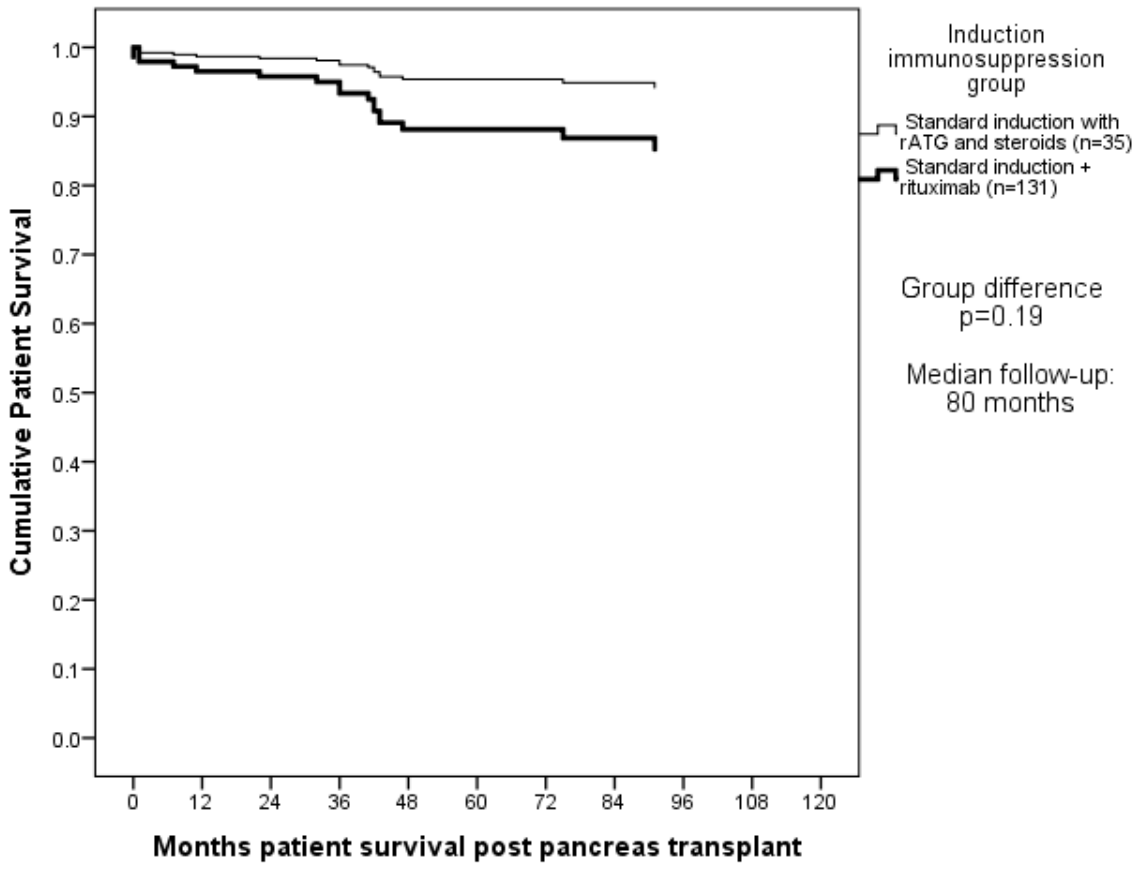


Figure 2



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