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ORIGINAL ARTICLE

Delayed introduction of sirolimus in paediatric intestinal transplant recipients: indications and long-term benefits

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SUMMARY

To review our experience using sirolimus in a single centre paediatric intestinal transplantation cohort. Intestinal transplant patients with more than 3 months follow-up were divided into two groups according to their immunosuppression regimen: tacrolimus, (TAC group, $n = 45$ grafts) or sirolimus (SRL group, $n = 38$ grafts), which included those partially or completely converted from tacrolimus to sirolimus. The indications to switch were tacrolimus side effects and immunological complications. Survival and complications were retrospectively analysed comparing both groups. SRL was introduced 9 months (0 months–16.9 years) after transplant. The main cause for conversion was worsening renal function (45%), followed by haemolytic anaemia (21%) and graft-versus-host-disease (16%). Both groups showed a similar overall patient/graft survival ($P = 0.76/0.08$) and occurrence of rejection (24%/17%, $P = 0.36$). Immunological complications did not recur after conversion. Renal function significantly improved in most SRL patients. After a median follow-up of 65.17 months, 28/46 survivors were on SRL, 26 with monotherapy, with good graft function. Over one-third of our patients eventually required SRL conversion that allowed to improve their kidney function and immunological events, without entailing additional complications or survival impairment. Further trials are warranted to clarify the potential improvement of the standard tacrolimus maintenance by sirolimus conversion or addition.

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Key words
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Aim/Background

Standard immunosuppression side effects and long-term immunological complications force to seek for alternatives in intestinal transplantation (IT). In the last decade, many publications have supported the benefits of sirolimus (SRL) and other mTOR inhibitors in solid organ transplantation, most of them focused

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on renal or liver transplantation [1–3]. However, despite their potential advantages, tacrolimus (TAC) monotherapy remains as the standard maintenance therapy [4, 5].

Literature addressing SRL benefits in paediatric IT patients is limited to small cohorts and case reports, often as a rescue therapy or for specific TAC-related effects in selected cases [6–9]. Some groups have compared results with TAC patients [10–14] and reported less rejection and higher survival rates in the short-term when SRL was added to TAC [9, 14]. However, few of them report its use as monotherapy and the results are limited to short-term outcomes [15–20]. The last report of the Intestinal Transplant Registry (ITR) considered SRL a good prognostic factor for patient and graft survival, for both paediatric and adult population [15, 21] but no further details were provided.

Our group reported minimal side effects and similar rejection episodes in a preliminary study of five patients, suggesting SRL could be a promising rescue therapy [22]. Forced by the need of facing the adverse TAC effects and encouraged by our previous experience, we markedly increased SRL use in our IT program over the last decade. In the present retrospective study, we found that almost 60% of the survivors in our series were on SRL monotherapy with normal graft function and no increase of adverse effects in the long-term, which has not been reported so far.

Patients and methods

Conversion to SRL

Criteria for conversion to SRL were divided into two groups: (1) TAC-related side effects and (2) immunological problems, these including steroid-refractory graft-versus-host disease (GVHD) (in which SRL benefits have been reported in allogeneic hematopoietic stem cell transplantation [23]) and lymphoproliferative disease (PTLD) or other tumours because of its antiproliferative action. Autoimmune disorders were also considered in the second group as bone marrow expression of GVHD [24].

Moderate nephropathy was defined as elevated creatinine (>1.5 mg/mL) and C cystatin (>1.6 mg/mL) and/or decrease in estimated glomerular filtration rate (eGFR)(<75 mL/min). Oedema, oliguria or hypertension developed only in severe cases.

The loading SRL dose was 2 mg/m^2 daily and adjusted to achieve blood levels in the 5–10 ng/mL range. SRL was introduced progressively, as TAC was tapered, with no changes on the steroid dose. Initially, blood levels were tested weekly and every 3 months thereafter. *Monotherapy* was defined as 'transplants in which the total conversion to SRL was achieved with the patient remaining on SRL for the rest of the follow-up'; Since the patients were converted to SRL at different times, the exposure time to SRL and TAC for each patient was considered in the statistical analysis.

Study design

After institutional review board authorization was obtained, transplants who were partially or completely converted to SRL over the follow-up (SRL group), were retrospectively reviewed and compared with those who stayed on TAC (TAC group). Intestinal grafts that did not survive more than 3 months after transplant were excluded from both groups to minimize selection bias.

Epidemiological data, type of transplant, preconversion immunological events (GVHD, PTLD, rejection and autoimmune disorders), time elapsed from transplant to SRL introduction and reason for switching to SRL were recorded. To determine possible genetic risk factors associated with TAC metabolism, the CYP3A5 polymorphism rs776746 A > G was analysed in the recipients using real-time PCR to identify fast, intermediate and slow TAC metabolizers (AA, AG and GG genotypes, respectively) [25, 26].

Resolution of TAC side effects and emergence of new ones were studied. Renal function was documented in patients with renal insufficiency just before the conversion and at the last follow-up by eGFR, through the CKD-EPI /Schwartz formula (based on creatinine levels) and Filler equation (based on cystatin C levels). Improvement in renal function was documented by decrease in C cystatin and creatinine levels or increase in eGFR >25%. The impact of the time elapsed between the transplant and conversion to SRL, age at transplantation, retransplantation and CYP3A5 polymorphism on renal improvement was also studied.

Serum triglyceride and total cholesterol levels at the last follow-up were compared between groups, since they can be affected by SRL [27, 28]. Additionally, liver and bone marrow function were measured periodically.

Resolution or new occurrence of immunological complications after conversion, which included acute and chronic rejection histologically confirmed, donor specific anti-HLA antibodies (DSA) appearance (DSA monitoring was performed since 2011 onwards), PTLD,

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GVHD/autoimmune disorders, were studied and compared with the TAC group. Since these complications could be cause or consequence for the conversion, we considered for the analysis if they happened while being on TAC, SRL or both.

Finally, patient and graft survival and retransplantation need were analysed comparing patients with or without SRL.

Statistical analysis

All data were expressed as mean ± standard deviation or median (minimum-maximum) and compared by treatment (SRL or TAC):

Chi-square tests were used to analyse the homogeneity in the variables: *number of IT performed before 2008, sex*, a*etiology of intestinal failure, type of graft*, *previous transplants*, *immunosuppression protocol and CYP3A5*.

A Generalized Linear Model (GLM) with the restricted maximum pseudo-likelihood method (RMPL) was performed with SAS Enterprise Guide 8.2 software to compare *months of follow-up, age at transplant, triglyceride* and *cholesterol* values, *renal function* variables and *immune complications*. The P-value from fixed effects was assessed with the type III test table.

For the renal function analysis, the averages of determinations between the conversion and the last follow-up date were calculated by least squares and compared: additionally, the model was adjusted to analyse the impact of time elapsed until conversion to sirolimus, CYP3A5, retransplantation and age at transplantation. These variables were introduced as simple fixed effects and were considered the random effect of the intercept.

A descriptive analysis of *immune complications* occurred for each transplant was performed over the follow-up *considering the date of the complication and if this happened being* with or without SRL. Thus, each complication was considered as a dependent variable and treatment (with or without SRL) was included as a fixed effect. The *probability of DSA appearance* by treatment and time (pretransplant, pre-SRL and post-SRL) was computed and compared with the Bonferroni test for multiple comparisons adjustment.

Survival analysis was performed with Stata v.14.0 Copyright 1985-2015 StataCorp LP, considering conversion to SRL as a time-dependent variable. Ties were handled with the Breslow method. The Hazard ratio (HR) was obtained using a Cox regression model.

P value >0.05 indicated no impact of treatment on survival. Two-sided tests were used.

Results

Intestinal transplant series

From 1999 to 2020, 108 IT (84 patients) were performed, at a median age of 3.1 years (0.5–30). The main indications were short bowel syndrome (65%), motility disorders (20%) and untreatable diarrhoeas (9.4%). The types of grafts were as follows: 21 isolated small bowel (ISBT), 26 combined liver-small bowel (CLST), 3 modified multivisceral (MMVT) and 58 multivisceral grafts (MVT). Retransplantation rate was 21%, with 24 additional grafts in 18 patients (6 required a third graft): 5 ISBT, 2 CLST and 17 MVT.

Tacrolimus plus steroids was the standard initial maintenance therapy, with a TAC dose of 0.05–0.1 mg/kg/12 h keeping within a range of 5–12 ng/ml from the third month post-transplant onwards. We used four different induction protocols, based on others' and our own personal experience (Table 1). Since 2008, the two most common protocols were alemtuzumab in retransplanted patients >4 years old, and basiliximab in younger patients.

Comparison between the SRL and TAC groups

SRL was used for the first time in 2008 in our series. Since then, 37 patients (39 grafts) have been converted to SRL, while 47 patients (69 grafts) have stayed on TAC for their entire follow-up or until death. Demographic and clinical data from both groups (excluding those with less than 3 months follow-up) are summarized in Table 2. Finally, 38 and 45 transplants were included in the SRL and TAC groups, respectively.

Sex, age at transplant and indication for transplantation were similar in both groups. Median time from transplant until SRL conversion was 9.1 months (0 months–16.9 years). Figure 1 shows the frequency and timing of conversion. Our policy was to not introduce SRL until 4 months after transplant, except for those who developed early TAC-related life-threatening complications ($n = 6$): two caused by hypertrophic cardiomyopathy, two caused by thrombotic microangiopathy, one caused by reversible leukoencephalopathy and one caused by severe haemolytic anaemia.

Sirolimus was used in all types of grafts although the distribution was significantly different than the TAC group ($P < 0.01$), with more presence of MVT and MMVT. This was consistent with the significantly higher retransplantation rate observed also in the SRL group compared with the TAC group (61.1% vs. 27.7%,

Table 1. Immunosuppression protocols.

	Immunosuppression protocol			
	Induction	Maintenance	n	Era
I	Basiliximab 4 doses (12 mg/m^2): POD 1 and 4, 1 month and 4 months	FK iv or po + steroids + AZA FK level target: • 15–20 ngr/ml first 2 w • 10–20ng/ml 2–4 w • 12–15ng/ml 4–8 w • 8–12 ng/ml thereafter Steroids: 10 mg/k/d the first day and tapering till • 2 mg/k/d 1 month • 1.5 mg 2–3 m • 1 mg 4–5 m • 0.5 mg/k from 8 month	14	1999–2004
II	Thymoglobulin 5 mg/kg, 2 doses: one pretransplant and one at POD 1.	FK FK level target: • 15- 20 ngr/ml first 2 w • 10–15 ng/ml 2–4 w • 10 ng/ml thereafter	17	2004–2005 /2014–2016
III	Basiliximab 2 doses (10 mg < 30 Kg, 20 mg > 30 kg)) on day 0 and 4 post-tx	FK + steroids FK level target: • 15–20 ngr/ml first month • 10-15 ng/ml 1-3 m • 8-10 ng/ml 3-12 m • 6-7 ng/ml thereafterSteroids: • 10 mg/k/d the first day and tapering till • 2 mg/k/d 1 month • 1 mg/k 2nd m • 0.5 mg 3rd m • 0.25 mg/k from 4th month	59	2006–2020
IV	Alemtuzumab 2 doses of (0.5 mg/kg). At transplant and at POD 4	FK FK level target: • 10-15 ngr/ml first 2-3 mo, • 5–10 ng/ml thereafter	18	2008–2014/ 2016–2020

$P = 0.04$), since MVT is preferred in our centre for retransplantation. The reasons for retransplantation were rejection (9) and PTLD (2) developed in an existing ISBT (10) or MVT (1) graft.

The induction protocols received were also significantly different from those in TAC group ($P < 0.01$). Although basiliximab was mostly received by both groups, the frequency was higher in SRL patients. Also, and related to the higher retransplantation rate, SRL group showed an increased use of alemtuzumab (protocol IV). In contrast, the earlier protocols I (basiliximab + azathioprine) and II (thymoglobulin) were barely used in SRL group but more frequent in TAC patients.

A multivariant analysis looking for differences between these variables (graft type, immunosuppression protocol and prior transplant) was not possible because of the small sample size in some groups.

Recipients' CYP3A5 polymorphism rs776746 A > G was analysed in 54/83 cases. The slow metabolizer genotype (GG) was detected in 44 transplants (72% from the TAC group and 89.6% from the SRL group), whereas the intermediate (AG) and fast (AA) metabolizer genotypes were respectively detected in 8 (20.0% from the TAC and 10.3% from the SRL group) and two transplants (both from the TAC group, 8.0%). No association was found between any genotype and the conversion to SRL ($P = 0.16$).

Conversion from TAC to SRL – indications and outcome

Tacrolimus side effects were the main reason for conversion in 65.8% ($n = 25$) of patients, with nephrotoxicity being the most frequent indication (44.7%, $n = 17$). In

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Table 2. Demographic and clinical data from TAC and SRL groups.

	SRL group	TAC group	$p^{¶}$
Transplants analysed (graft survival >3 months)	38	45	
Average follow-up*	65.17 +/-8.48 months (23d–11.9 years)	41.7 +/-5.73 months (0 d- 20.8 years)	0.02
Number of it performed before 2008	11/38 (29%)	25/45 (56%)	0.03
Sex (Male/Female)	19 /17	24/15	0.50
Median age at TX (Years)	3.3 (0.5–30.5)	3 (0.7–19.7)	0.24
Etiology of intestinal falure†	21 SBS, 10 MD, 3 UD, 2 tumour, 2 others	29 SBS, 10 MD, 5 UD, 1 tumour	0.47
Graft type‡	3 ISBT; 4 CLST; 29 MVT; 2 MMVT	15 ISBT; 14 CLST; 15 MVT; 1 MMVT	<0.01
Previous transplants	11 (61.1%)	5 (27.7%)	0.04
Induction protocol§	I (2); II (1); III (25); IV(10)	I (8); II (10); III (23); IV(4)	<0.01
CYP3A5 Polymorphism	26 GG, 3GA, 0 AA	18 GG, 5GA, 2 AA	0.16

*Follow-up data were available in 100% of the transplants

†SBS: Short bowel syndrome; MD: Motility disorders; UD: Untractable diarrhoea.

‡ISBT: isolated small bowel transplant; CLST: combined liver-small bowel transplant; MVT: multivisceral transplant; MMVT: modified multivisceral transplant

§Detailed in Table 1

¶A multivariant analysis was not possible because of the small sample size in some groups: for instance, only five patients had a prior transplant in the TAC group; the frequency of nonmultivisceral grafts was very low in the SRL group, and only 1 patient received a MMVT in the TAC group.

seven of these patients, renal insufficiency was accompanied by an immune complication, which added an argument for the conversion. Other TAC side effects were thrombotic microangiopathy, cardiotoxicity, allergies and neurotoxicity (Table 3).

Renal function improved in 15/17 patients who had previously suffered a worsening of kidney function (Fig. 2). However, two patients did not recover despite conversion. The first one received SRL in the context of severe GVHD that required high doses of several immunosuppressants that worsened her renal function until being currently in waiting list for kidney transplantation. The second patient lost her graft because of liver rejection and temporarily returned to TAC again after retransplantation, worsening her already weakened kidney function. She is currently stable on SRL but with a GFR of 30 ml/min/1.73m^2.

Neither the CYP3A5 genotype, previous transplants nor age at the time of transplantation influenced the renal function improvement ($P > 0.05$). However, creatinine levels and eGFR improved more in patients who were converted later than in those who were converted earlier ($P = 0.03$ and $P = 0.0008$), without finding significant differences in cystatin or in Filler´s equation ($P = 0.14$ and $P = 0.05$, respectively).

In addition to the seven patients with additional renal insufficiency, in another 13 patients (34.2%) the occurrence of *immunological complications* was the reason of conversion. In six of them (15.8%), SRL was used as rescue therapy for steroid-resistant GVHD, since its benefits have been reported in hematopoietic transplantation [29]. Autoimmune disorders such as haemolytic anaemia ($n = 8$, 21%) and pancytopenia/neutropenia ($n = 2$, 5.3%) were considered also an indication for conversion as they could be an early bone marrow manifestation of GVHD [30]. In four more patients (10.6%), SRL was used to treat PTLD because of its antiproliferative properties.

All haemolytic anaemia, autoimmune pancytopenia and PTLD resolved and did not recurred in any of the patients converted to SRL, after receiving their respective treatments (steroids boluses for the cytopaenia and Rituximab and gammaglobulin for PTLD), being all of them alive at the end of the study. Regarding patients converted because of GVHD, 50% are alive and did not present this complication again. The other 50% died because of its progression (two of them died within 3 months of conversion not being their evolution attributable to SRL). Comparison with the TAC group showed no greater risk of developing immunological complications (acute, humoral or chronic rejection,

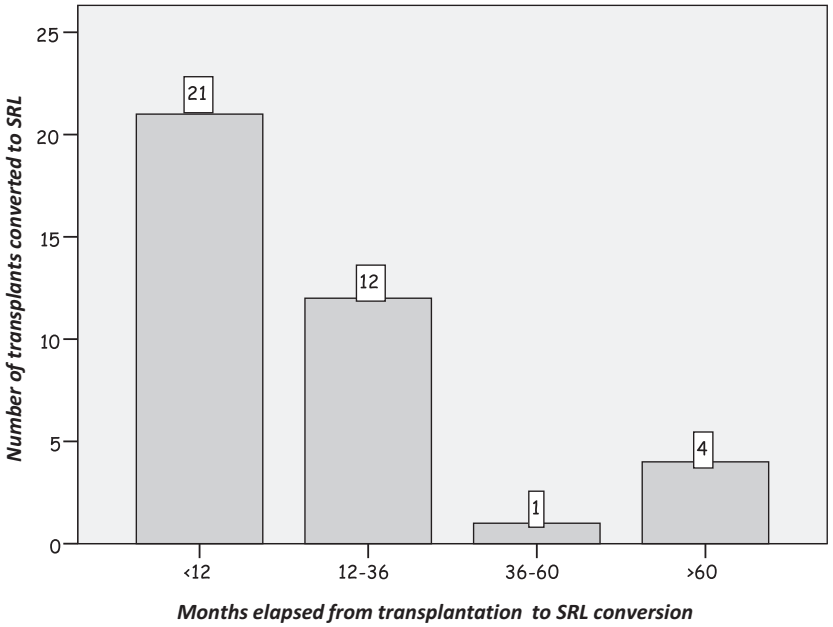


Figure 1 Survival curve style presentation to depict the frequency and timing of changes of SRL conversion.

DSA appearance, GVHD, autoimmune disorder or PTLD) or need for retransplantation after the conversion to SRL ($P > 0.05$, Table 4).

Morbidity and mortality SRL-related side effects

After a median follow-up of 65.17 months (1 month–12 years), no adverse effects have been detected related to SRL. Average triglyceride and cholesterol serum values at last follow-up were slightly higher in the SRL group (127.4 ± 14.5 and 174.8 ± 8.21 mg/dL) than in the TAC group (69.11 ± 19.9 and 131.8 ± 11.19 mg/dL) ($P = 0.25$ and $P = 0.19$, respectively), with no additional treatment required.

Rejection rate and DSA development before and after conversion

Twenty-seven patients (75%) did not develop any immune complication after conversion, whereas nine were diagnosed with rejection (except for two mild rejections, all cases are summarized in Table 5). Six of them already had a medical history of rejection prior to conversion to SRL, and even four had been retransplanted for this reason. SRL was added after receiving their second or third graft to slow the decline in kidney function; however, two of them died because of to the progression of a new chronic rejection, one is on the waiting list and one is currently stable on combined therapy but will probably be included soon for a third graft. Interestingly, despite good small bowel function, two patients developed late liver rejection: one was successfully retransplanted with a MVT and the other is awaiting a liver transplant with recurrent cholangitis episodes.

Finally, one patient died of sepsis on the waiting list for a second graft because of rejection, being on combined therapy because of renal impairment.

Comparison with the TAC group showed no greater risk of developing rejection after the conversion to SRL ($P > 0.05$, Table 4).

Regarding DSA within the SRL group, three retransplanted patients showed preformed DSA and four more patients developed DSA (de novo or preformed) after their first transplant. Five of them cleared their DSA after SRL conversion, but three new patients developed DSA being on SRL (Fig. 3). No significant differences were found in the frequency of *de novo* DSA development when compared with the TAC group (Table 4).

Patient and graft survival

At the time of this study (April 2020), 46/84 patients are alive, (55% of the global series), with a median age of 11 years (3.25- 36.25 years). Twenty-eight out of the initial 36 patients are on SRL (30 grafts; median age 14 years, range 4–36 years) and 18 remain on TAC (median age 13 years, range 3.3–30.6 years). One patient was converted to SRL but returned to TAC and MMF after being retransplanted in a different centre.

Table 3. Indications for SRL conversion/addition and outcomes in the long-term.

Indications for conversion/addition		n (%)	Outcome	Recurrence	Survival
TAC side effects (25; 65.8%):	Renal insufficiency +/- hypertension	17; 44.7%*	Improved in 15	No	15 (88.2%)†
	Thrombotic microangiopathy	3; 7.9%	100% resolved	No	2 (66.7%)†
	Cardiotoxicity (hypertrophic cardiomyopathy	2; 5.3%	100% resolved	No	2 (100%)
	Multiple allergies	2; 5.3%	100% improved	No	2 (100%)
	Neurotoxicity (reversible posterior leukoencephalopathy)	1, 2.6%	100% resolved	No	1 (100%)
Immune complications (20; 52.7%)*	Autoimmune haemolytic anaemia	8; 21%	100% resolved	No	7 (87.5%)†
	GVHD	6; 15.8%	50% resolved	No	3 (50%)†
	PTLD	4; 10.6%	100% resolved	No	4 (100%)
	Neutropenia/pancytopaenia	2; 5.3%	100% resolved	No	2 (100%)

*10 alone; 7 patients were converted because of both reasons (renal insufficiency and immune complication).

†7 patients died, all of them because of immune reasons (GVHD, progression of rejection or sepsis in the context of rejection) independently of the reason for conversion.

Despite all patients under SRL started their conversion with combined therapy, the 92% of living recipients (26/28) are on monotherapy with no adverse effects. The remaining two are currently on combined therapy: one awaiting a third graft and the other still in the process of conversion.

Overall patient survival was similar in both groups ($P = 0.76$). However, graft survival resulted higher in the SRL than in the TAC group as shown in Fig. 4a, although this was not statistically significant ($P = 0.08$). We found a similar patient and graft survival rate in patients converted to SRL for immune reasons than in those with TAC-related complications when compared with the TAC group ($P = 0.93$ and $P = 0.22$) (Fig. 4b).

Seven patients died while on SRL: two developed multiple immune and post-transplant complications, with SRL used as a rescue therapy, and follow-up was not sufficient to confirm the safety or efficacy of SRL. The other five died of sepsis (1, on combined therapy), chronic rejection progression (2, both re-transplanted patients receiving combined therapy) and refractory GVHD (2) (Table 3).

Discussion

The side effects of the standard TAC immunosuppression have become one of the major complications during long-term survival [9] after solid organ transplantation. In the last two decades, mTOR inhibitors emerged as a good alternative in kidney [1, 5, 31–33], liver [2, 4, 34], heart [35, 36] and even IT [7, 8, 13], mostly used to prevent TAC-related nephrotoxicity. Besides, SRL arose the attention of research in the field of operational tolerance, given their immunomodulatory and antiproliferative properties [37]. Unlike Tacrolimus, SRL does not only inhibit T cell proliferation but also modulates regulatory T cells and dendritic cells differentiation, activation and function. Additionally, benefits for PTLD, GVHD, autoimmune cytopaenia and rejection have been reported [24, 29, 30, 38].

Despite these promising evidences, there are no recent reviews about SRL therapy in IT and even less information in paediatric recipients or as monotherapy in the long-term. This manuscript presents the largest IT series to date of children converted partially or completely from TAC to SRL. In accordance with the last ITR report, we found a trend to improved graft survival in the SRL group, although no differences were found in patient survival [15, 21]. This beneficial effect of SRL was more evident in those patients converted because of TAC-related complications but also present in those

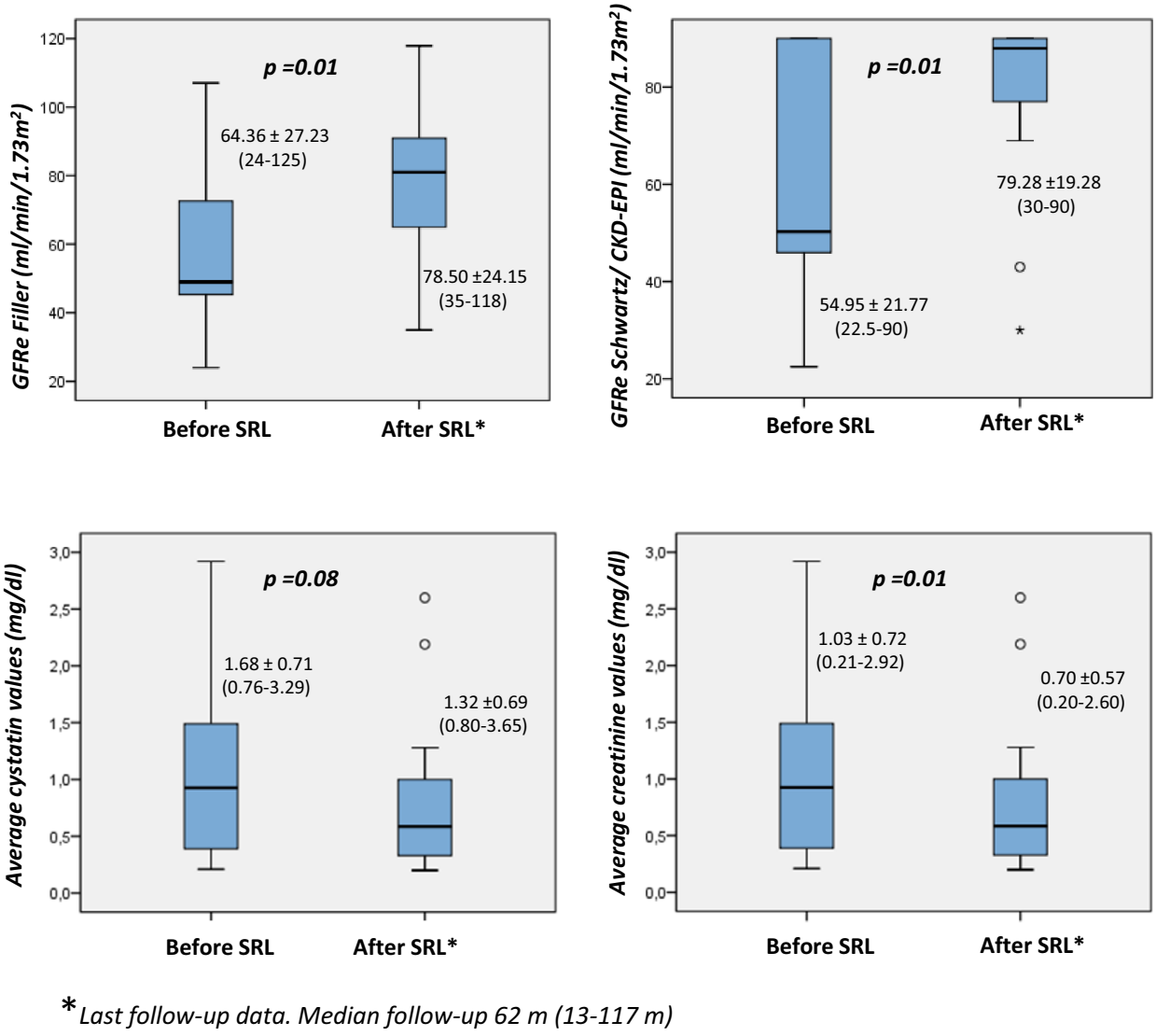


Figure 2 Renal function prior and after conversion (last follow-up) in patients converted to SRL because of renal insufficiency. Values expressed in mean ±standard deviation (range minimum-maximum).

with immunological reasons. This is an important finding as it encourages the use of SRL even in patients without impaired renal function. Interestingly, PTLD, GVHD or autoimmune cytopaenia resolved and did not recur after conversion, thus supporting the widely reported immunomodulatory properties of mTOR inhibitors [38, 39].

One of the main barriers to SRL conversion has been the higher rejection rates reported in earlier conversion trials [40]. Despite some reports encourage to add SRL to TAC to prevent rejection, there are other authors reporting the opposite findings. However, our incidence of rejection in the SRL group was similar than in those on TAC. Even more so, some of these patients had had earlier rejections with TAC, being retransplanted for that reason. Differently from those of the TAC group, three rejections within SRL group were limited to the liver, suggesting a different type of rejection, something that should be clarified with additional clinical and experimental studies [41, 42]. Regarding the development of *de novo* DSA, we found like others did previously [43] no significant differences between groups, although our analysis is limited by some incomplete data.

In almost half of the cases, the reason for conversion was the progressive worsening of renal function, which improved markedly in most of them. This is especially important in a paediatric series with a longer life expectancy. From a retrospective point of view, in the two patients in our series in whom renal function did not

Table 4. Comparative risk for appearance of immune complications and need for retransplantation after conversion compared with TAC group.

	n	SRL group (38 grafts)	Tac group (83 grafts)*	*P*
Acute rejection episodes	23	9 (23.6%) (2 mild, 3 mod[†], 4 severe)	14 (16.87%) (3 mild, 5 mod, 7 severe)	0.36
Chronic Rejection	7	3 (2 retransplanted, one died listed)	4 (100% of them retransplanted, and 3 converted to SRL later)	0.50
Humoral rejection	2	1	1 (converted to SRL)	0.58
DSA appearance[‡]	16	3	13	0.24
Retransplantation	16	4 (10%) (2 being only on SRL, 2 being on combined therapy)	12 (14.4%)	0.57
GVHD	16	0	16 11 converted to SRL[‡], 45% exitus; 5 not converted, 100% exitus	–
Autoimmune disorders	11	0	11 8 converted to SRL[§], 100% alive 3 not converted, 33% exitus	–
PTLD	12	0 (SRL as rescue therapy, exitus)	12 7 converted to SRL[§], 100% alive 5 not converted, 80% exitus	–

*All transplants are included here, those who stayed on TAC over the entire follow-up, and those from the SRL but before the conversion.

[†]Two grafts developed acute moderate rejection and chronic or humoral rejection at different moments of the follow-up.

[‡]Analysed since 2011.

[§]Part of these GVHD, PTLD or AHAI happened in the past, and were successfully treated before conversion, in other cases conversion to SRL was part of the treatment.

improve, tacrolimus should have been withdrawn earlier before irreversible damage developed. Currently, our policy is to add SRL as soon as renal function begins to worsen and although we have more retransplanted patients with severe kidney disease, this has not been irreversible in any case. As confidence with SRL increase, hopefully we will be able to identify early risk factors and adjust the optimal treatment in an individualized manner. In this sense, we have addressed the possible correlation between the development of TAC-side effects and the recipients' CYP3A5 polymorphism rs776746 A > G, finding no evidence as a risk factor. However, since CYP3A5 is mainly expressed in intestine and liver, donors' genotype should be also analysed especially in those patients with MVT and CLST [44, 45] (not possible in the present study because of unavailability of donors' DNA).

Some other limitations derived from the study design must be considered. The first one is that comparative results do not involve two different cohorts of transplants (TAC vs. SRL). They all started on TAC and only some of them were converted to sirolimus. Additionally, as this is a 20-year retrospective study, different approaches have been used over the years and modified based on the experience. In fact, when we presented our results in 2015 (CIRTA; Buenos Aires, data not published), the 50% of the 23 patients who were on SRL were also receiving TAC, to prevent immunological complications. The other 50% were on monotherapy, after developing a TAC-related life-threatening complication. Over time, a faster and earlier transition from combined therapy to monotherapy has occurred in our series, encouraged by the good results obtained with a fairly long follow-up and the difficulties of registering two immunosuppressants when level monitoring [39]. In fact, 92% of our living patients on SRL are nowadays on monotherapy. Obviously, this makes difficult the comparison, although the exposure time to both immunosuppressants for each patient was considered in the analysis.

Another limitation is that SRL was introduced in 2008, and many patients who are still on TAC are from the first decade of our program, with less experience of the centre in facing post-transplant problems, which could affect favourably on SRL results when comparing both groups. In that sense, the exclusion of the grafts lost in the first three months post-transplant, more frequent during the first era, tried to minimize

Table 5. Rejection episodes after the conversion to SRL (only moderate and severe rejection, mild not included).

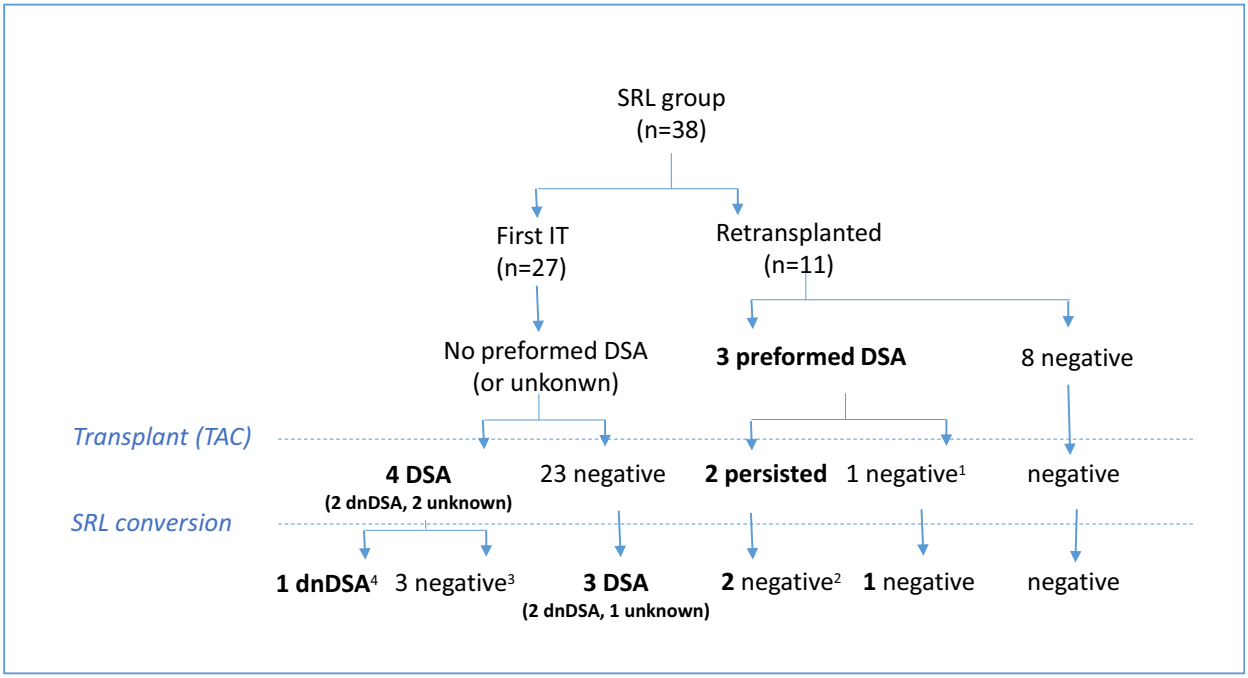
	Type of rejection	Time from transplant	Time from conversion	Past medical history of rejection	Observations	Survival
Pt. 1 (MVT)	Moderate acute rejection	11 months	10 months	NO	Responded well to steroids	Alive
Pt. 2 (ISBT)	Chronic rejection	14 years	10 years	NO	Retransplanted (MVT) and returned to SRL monotherapy	Alive
Pt. 3 (CLST)	Liver rejection	8 years	6 years	NO	Retransplanted (MVT) and returned to SRL monotherapy	Alive
Pt. 4 (MVT)	Liver rejection	8 years	6 years	2 prior grafts (ISBT and MVT)	Listed for liver transplantation	Alive
Pt. 5 (ISBT)	Chronic rejection	10 years	2 years (combined therapy)	Humoral rejection 8 years after ISBT	Retransplanted (MVT), reconverted to TAC and MMF	Alive
Pt. 6 (ISBT)	Chronic rejection	7 years	6 years (combined therapy)	2 prior grafts (2 ISBT)	Retransplanted (MVT)	Exitus
Pt. 7 (MVT)	Chronic rejection, IBD like	7 years	6 years (combined therapy)	3 prior grafts (2 ISBT and 1 MVT)	Progression of the chronic rejection and renal failure	Exitus
Pt. 8 (MVT)	Chronic rejection, IBD-like	7 years	6 years (combined therapy)	2 prior grafts (ISBT and MVT), resection of the last part of the graft (IBD-like)	Will probably require third transplant	Alive
Pt. 9 (MVT)	Acute and chronic rejection	3 years	2.8 years (combined therapy)	Liver rejection, chronic rejection	Died listed for retransplantation because of to sepsis	Exitus

this bias. In our cohort, retransplantation also appears as a possible confounding factor related with higher frequency of MVT and MMVT, as well as with the induction with alemtuzumab, all of them important confounding factors in the graft outcome. The limited number in the subgroups of patients after stratification makes the statistical comparison and clinical interpretation of findings challenging. Therefore, caution needs to be taken when applying such data prospectively or to clinical application. A multivariate step-forward modelling will be beneficial to address if the addition/conversion of SRL had an independent role in the patients' long-term survival, but this would be limited by the sample size. However, this heterogeneity also revealed interesting findings. For example, the higher rate of patients with a complex medical history (such as previous transplants and/or immunological complications before conversion) in the SRL group did not interfere with their good long-term results, being the survival similar to or even better than that of those thought to be 'easier' patients.

In summary, this is the first study describing promising outcomes of long-term survivors in the largest paediatric IT cohort with maintenance immunosuppression based exclusively on SRL and low-dose corticosteroids. We have gradually gained confidence with its use, especially in younger children, complex settings, immunological problems, and those with less time elapsed from transplant, findings that could potentially encourage a similar approach in other groups. Translational research as well as multicentre randomized clinical trials would help to determine the appropriate indications, timing and schedule for the combined use or conversion to SRL in IT recipients.

AUTHORSHIP

Ane M. Andres (Andres AM): Conception and design of the study; acquisition of data; analysis and interpretation of data; Wrote the article and revised it critically for important intellectual content. **Paloma Talayero (Talayero P):** Revised the article critically for important intellectual content. Important contribution to the CYP3A5 and DSA data. **Alida Alcolea-Sanchez (Alcolea-Sanchez A):** Adquisition of clinical data. Clinical management of the patients. **Alba Sanchez-Galán (Sanchez-Galan A):** Adquisition of clinical data. Clinical management of the patients. **Javier Serradilla Rodríguez Javier (Serradilla J):** Adquisition of clinical data. Clinical management of the patients. **Alba Bueno Jimenez Alba (Bueno A):** Adquisition of clinical data.



[1]*Treated with rituximab for PTLD;* [2] *Treated with rituximab for PTLD, and intravenous immunoglobulin for a severe hypogammaglobulinemia;* [3]*One cleared after desensitization protocol and 2 spontaneously;* [4] *Developed chronic rejection*

Figure 3 DSA within the SRL group prior to transplant, after transplant before conversion to SRL and after conversion to SRL (monitoring every 6 months since 2011 onwards); values were considered positive when anti-HLA class I standard fluorescence intensity (SFI) was >15,000 and anti-HLA class II SFI was >20,000 [46].

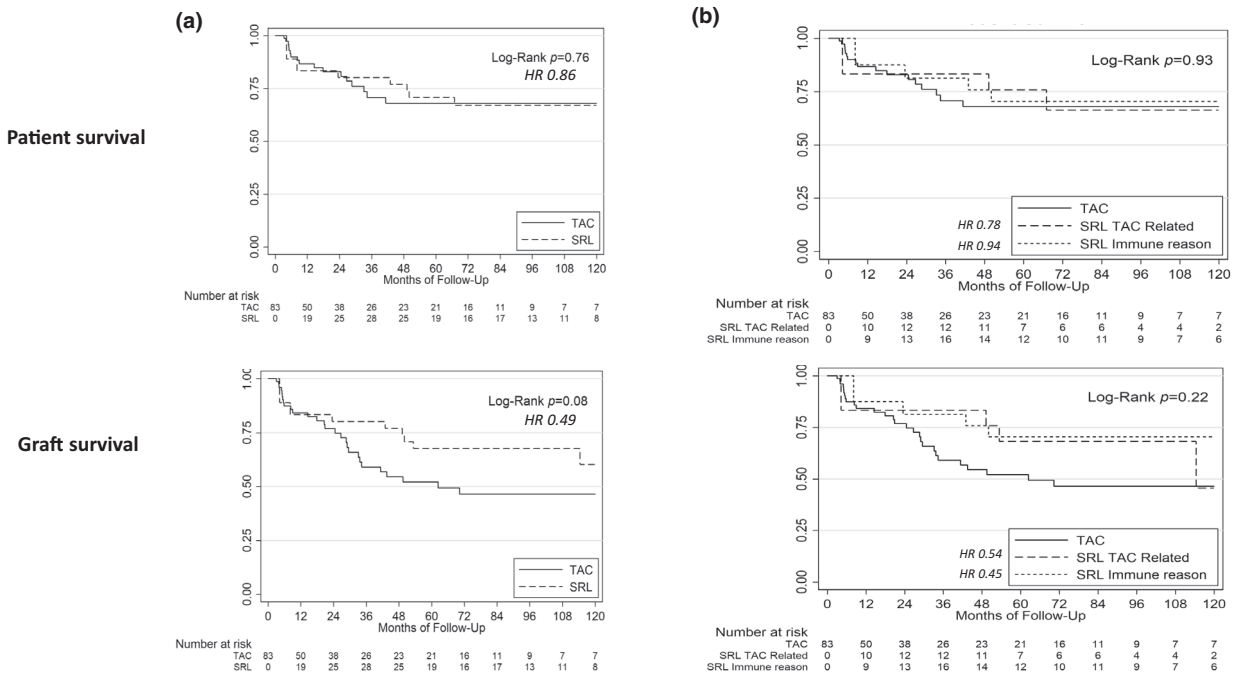


Figure 4 Patient and graft survival (a); Patient and graft survival considering the reason for conversion (b). This is a time-dependent survival. The 83 cases started with TAC; subsequently, three different scenarios may occur: that they change their treatment (from TAC to SRL), that they suffer some event (exitus or graft loss) or none of these until the last follow-up.

Clinical management of the patients. **Rocío Gonzalez Sacristan (González-Sacristán R)**¨ Adquisition of clinical data. Clinical management of the patients. **Pablo Stringa (Stringa P):** Revised the article critically for important intellectual content. **Rodrigo Papa-Gobbi (Papa-Gobbi R):** Revised the article critically for important intellectual content. **Maria Lasa Lazaro (Lasa-Lazaro M)** ¨ Important contribution to the CYP3A5 and DSA data. **Mariana Díaz –Almirón (Diaz-Almiron M):** Important contribution to the statistical analysis. **Esther Ramos Boluda (Ramos-Boluda E):** Revised it critically for important intellectual content and final approval of the version to be submitted. **Manuel Lopez-Santamaría (Lopez-Santamaria M):** Revised it critically for important intellectual content and final approval of the version to be submitted. **Francisco Hernández-Oliveros (Hernandez-Oliveros F):** Conception and design of the study. Revised it critically for important intellectual content and final approval of the version to be submitted.

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CONFLICT OF INTEREST

All the authors declare no conflicts of interest.

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