

TARGETED IMMUNOSUPPRESSION WITH GRAFALON

A Handbook on Clinical Evidences from India

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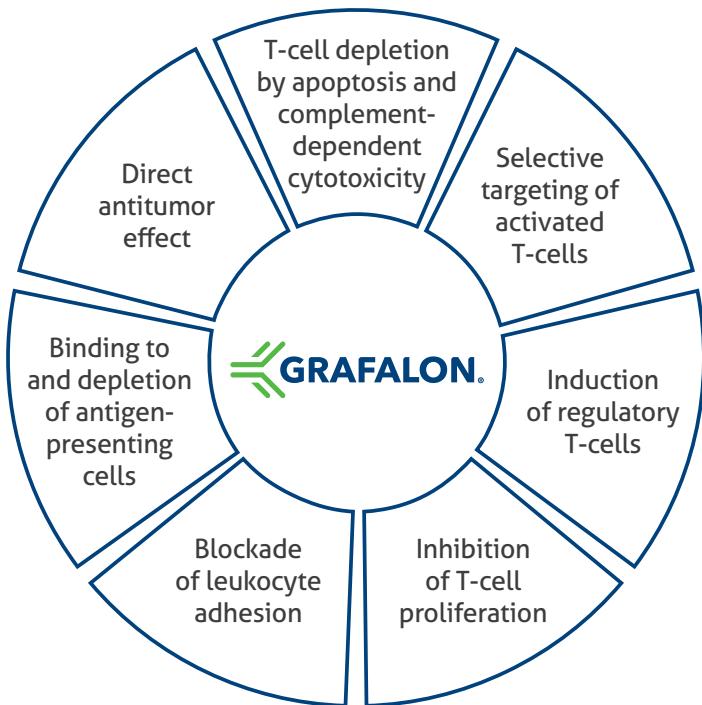
Grafalon: Introduction

Grafalon/ATLG, formerly known as antithymocyte globulin-Fresenius or ATG-F, is a potent immunosuppressive polyclonal T-lymphocyte-depleting agent used for the prevention and treatment of graft rejection in a patient undergoing transplant. It is produced by immunizing rabbits with a homogenous human T-lymphoblastoid cell line, Jurkat.^{1,2}

Grafalon is approved for use in >50 countries worldwide, including India (introduced in 2016).^{2,3}

Mechanism of Action²

The primary mechanism of action of ATLG is the depletion of immune cells, specifically activated T-cells. However, other immunomodulatory properties are also involved. The biological activity of ATLG is mediated by antibody specificities and has been shown to include a variety of possible mechanisms of action.



Pharmacokinetic Properties²

Bioavailability	100%
Distribution	Plasma and extravascular fluid
Half-life	14 days (approx.)

The serum concentrate contains 20 mg/mL of anti-human T-lymphocyte immunoglobulin from rabbits.

Elimination of Grafalon occurs either by binding to a target and the subsequent phagocytosis or by degradation.

Grafalon undergoes protein metabolism, with no non-physiological metabolites known to exist.

Dosage and Indications²

Table 1 summarizes the approved indications of Grafalon in Europe, which includes stem cell transplantation and solid organ transplantation.

Table 1: Indications and recommended dosage regimens

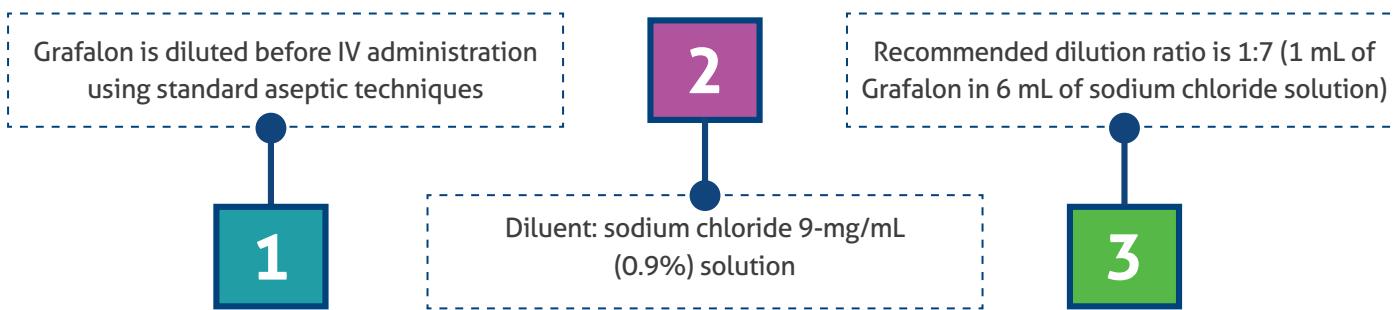
Indication	Recommended dose	Infusion time	Duration	Total dose
Prophylaxis in stem cell transplantation	20 mg/kg/d	4–12 hours	Days –3 to –1 before transplant	60 mg/kg
Prevention of acute rejection in solid organ transplantation	2–5 mg/kg/d	4 or 0.5–2 hours intra-operatively	Initiated on day of transplantation pre-, intra-, or immediately postoperatively 5–14 days	10–70 mg/kg ^a
Treatment of acute rejection in solid organ transplantation	3–5 mg/kg/d, commonly 3–4 mg/kg/d	4 hours	5–14 days	15–70 mg/kg ^a

^aThis recommended dosing regimen of Grafalon is on the higher side, as it was approved during cyclosporin era. However, in real-world settings, dosing regimens may vary between countries and clinicians are advised to refer to the respective national approved labeling.

In India, the average cumulative dose of Grafalon used in low to intermediate immunological risk patients is ~6 mg/kg body weight.⁴

In high immunological risk patients, the average cumulative dose of Grafalon used is 8–10 mg/kg.⁵

Dilution/Preparation⁶

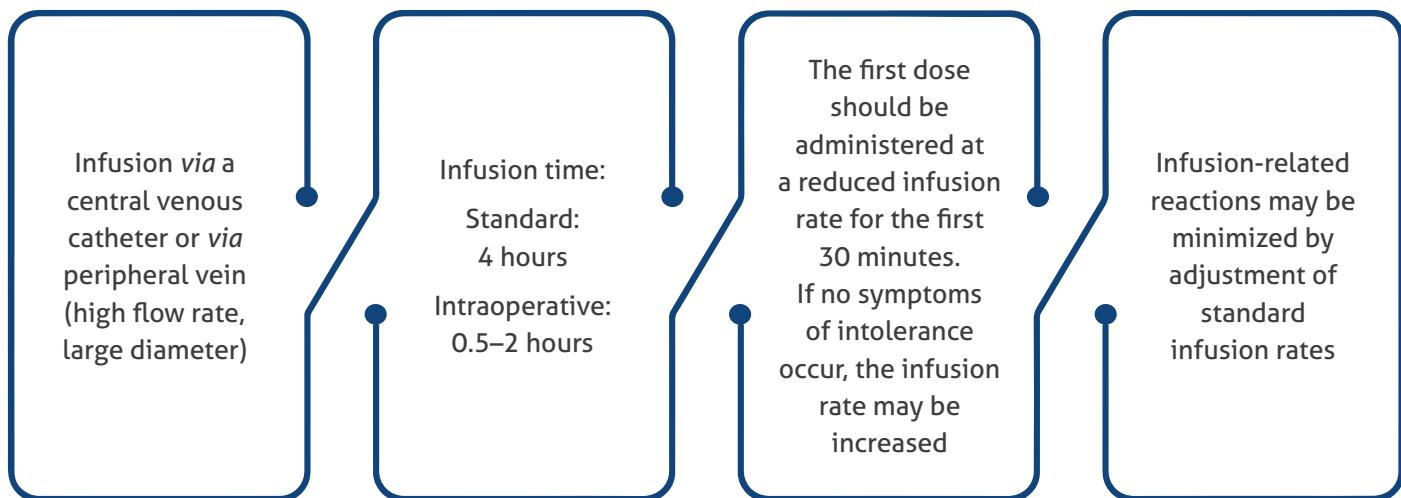


CAUTION: Grafalon should not be mixed with glucose, blood, blood derivatives, solutions containing lipids, or sodium heparin

Premedication²

Steroids & antihistamine are given before administering Grafalon to improve systemic & local tolerance.

Methods of Administration⁶



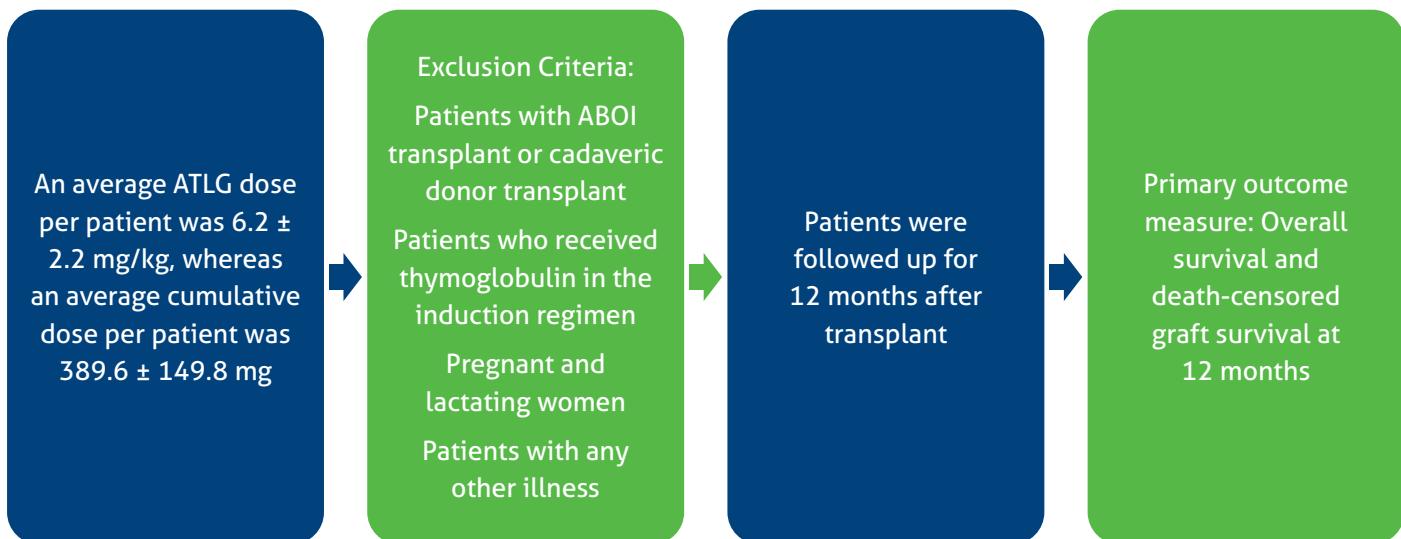
Indian Experience with Grafalon/ATLG: Structured Summary of Clinical Studies

1. One-year outcomes with use of anti-T-lymphocyte globulin in patients undergoing kidney transplantation: Results from a prospective, multicentric, observational study from India⁴

Gang *et al* (2022) presented the results of a prospective, multicentric, observational study that included 359 patients (aged ≥ 18 years) with ESKD undergoing KT and who were prescribed Grafalon (ATLG) (as a part of induction).

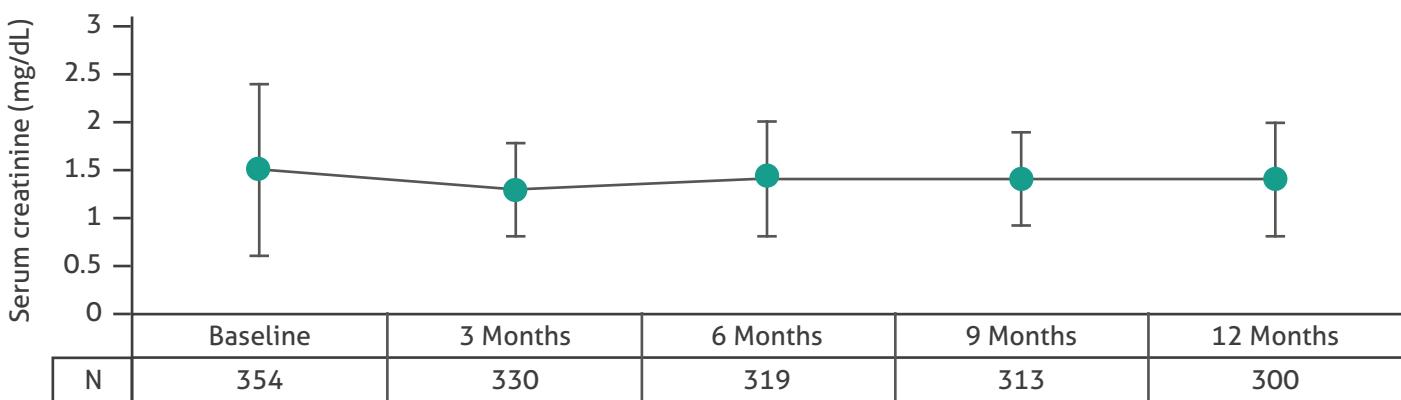
Study Design

- This study was conducted across 11 centers in India making it the largest cohort of Indian patients in kidney transplantation.



Efficacy and Safety Results

- During a 12-month period, the graft function was well preserved (Figure 1).



mg/dL, Milligrams per deciliter.

Figure 1: Changes in serum creatinine for 12 months

- The rate of graft dysfunction was observed in 13.4% of patients, whereas 6.7% of patients had BPAR.
- In total, 5.3% and 1.1% of patients reported ACR and ABMR, respectively, whereas one patient had both ACR and ABMR (Table 2).
- In 1.4% of patients, calcineurin inhibitor toxicity was responsible for graft dysfunction, and 1.4% of patients showed asymptomatic rise in creatinine level (Table 2).

Table 2: Transplant outcomes

Parameters	Observations
Graft dysfunction	48 (13.4)
BPAR	24 (6.7)
ACR	19 (5.3)
ABMR	4 (1.1)
ABMR + ACR	1 (0.3)
Calcineurin inhibitor toxicity	10 (2.8)
Graft dysfunction conservative management	5 (1.4)
Acute tubular necrosis with cortical necrosis	4 (1.1)
Asymptomatic rise in creatinine	5 (1.4)
Infective episodes	49 (13.6)
Urinary tract infection	25 (7.0)
Sepsis	12 (3.3)
Lower respiratory tract infection	4 (1.1)
Cytomegalovirus disease	1 (0.3)
Varicella-zoster virus disease	1 (0.3)
Others ^a	6 (1.7)
Deaths	12 (3.3)
Graft loss	1 (0.3)
Patient survival after 12 months	96.65%
Death-censored graft survival	99.44%

ABMR, Antibody-mediated rejection; ACR, Acute cellular rejection; BPAR, Biopsy-proven acute rejection.

^aOthers: graft pyelonephritis ($n = 1$), perigraft collection ($n = 1$), gastroenteritis ($n = 1$), osteomyelitis ($n = 1$), right gluteal abscess ($n = 1$), perianal abscess ($n = 1$).

- The overall survival and death-censored graft survival at 12 months were 96.65% and 99.44%, respectively.
- A total of 13.6% of patients developed one or more infection, where UTI was the most common infection (7%) followed by sepsis (3.3%).
- Graft loss was observed in one patient after ABMR.
- The all-cause mortality was reported as 3.3%.

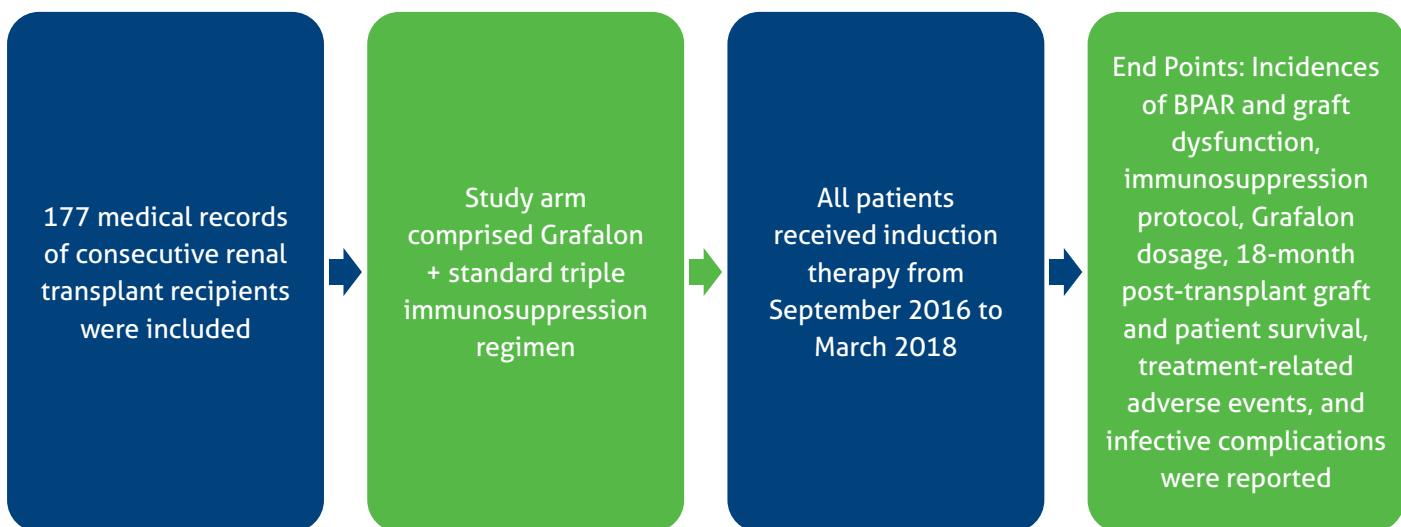
Key Outcomes

- Grafalon shows a favorable impact on the survival and graft function in ABO-compatible Indian renal transplant patients.
- Grafalon at an average dose of 6 mg/kg is a safe induction regimen immunosuppressant for ABO-compatible Indian renal transplant patients.

2. Anti-T-lymphocyte immunoglobulin (Grafalon) as an induction agent for renal transplantation: A real-world, retrospective, single-center experience⁷

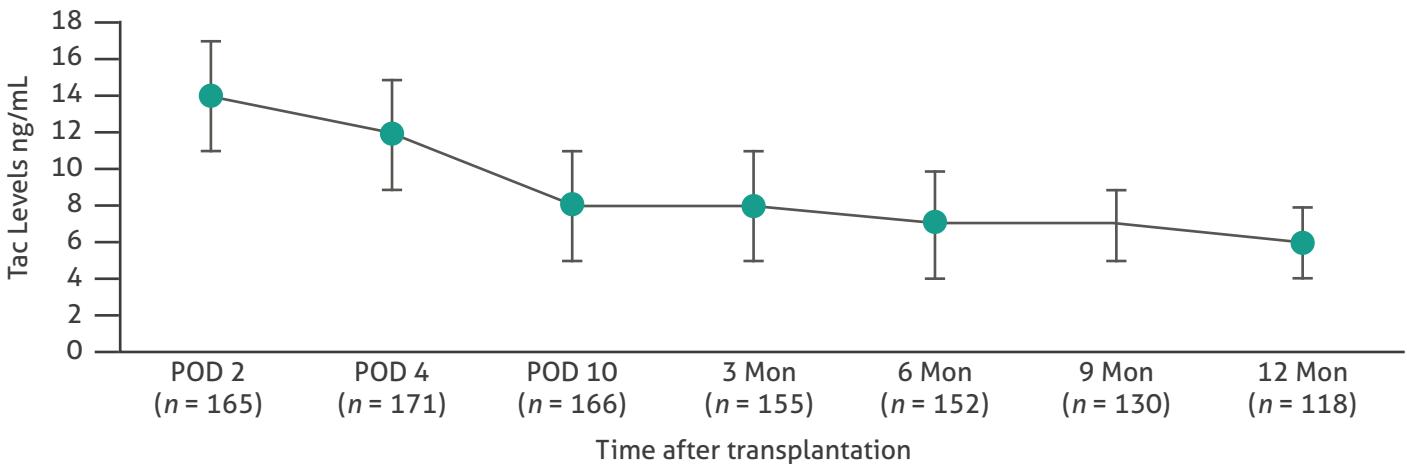
Gupta *et al* (2022) conducted a retrospective, single-center, observational study to report the clinical experience from the real-world use of Grafalon (ATLG) as an induction agent in renal transplant recipients from India between September 2016 and March 2018. In this study, 177 medical records of consecutive renal transplant recipients were included.

Study Design



Efficacy and Safety Results

- Tacrolimus dose adjustments maintained the level of tacrolimus at 8 to 9 ng/mL until 1 month after transplant, which was then reduced to 6-7 ng/mL afterward (Figure 2).



Tac, Tacrolimus; POD, Postoperative day

Figure 2: Mean tacrolimus levels in study renal transplant recipients who received Graffalon as induction agent

- The mean Graffalon dose used in the study group was 5.81 ± 1.95 mg/kg, whereas the minimum and maximum doses were 2.41 and 10.07 mg/kg, respectively (Table 3). Additional dose of Graffalon was not given if the ALC dropped below 200/uL.

Table 3: Graffalon dose details for study renal transplant recipients

Characteristics	Result (N = 177)
Average dose per patient, mg/kg	5.81 ± 1.95
Cumulative dosage per patient (range), mg	349.06 ± 117.15 (200–900)
Induction with <4 mg/kg	27 patients
Induction with 4–8 mg/kg	134 patients
Induction with >8 mg/kg	16 patients

mg/kg, Milligrams per kilogram.

- The mean creatinine values remained low even after discharge (1.24 - 1.36 mg/dL) during follow-up visits till 12 months after transplant (Table 4).
- Graft dysfunction was reported in 26 patients (14%), of them 11 patients (6.2%) had BPAR, 11 patients (6.2%) had acute tubular necrosis, and 4 patients (2.2%) had calcineurin inhibitor toxicity.

Table 4: Changes in serum creatinine levels for 12 months in study renal transplant recipients who received Grafalon as an induction agent

	Baseline	Discharge	3 months	6 months	9 months	12 months
No. of patients	177	176	170	169	161	161
Mean creatinine ± SD, mg/dL	8.75 ± 3.03	1.24 ± 0.54	1.30 ± 0.33	1.36 ± 0.33	1.34 ± 0.64	1.36 ± 0.35

mg/dL, Milligrams per deciliter; SD, Standard deviation.

- Seven deaths (3.9%) were reported in the study group, and the causes of death included fungal pneumonia, bacterial pneumonia, and acute coronary syndrome in two patients and UTI with septicemia in one patient.
- Death-censored graft survival was 100% and 98% till month 12 and month 18 of follow-up, respectively.
- The overall patient survival was 96% until the end of the study.

Key Outcome

The outcome from this real-world evidence supports the safe and effective use of Grafalon as an induction agent in renal transplant recipients with an individualized dosing approach.

3. Comparison of thymoglobulin and Grafalon as induction agents in renal transplantation: A prospective study⁸

Thukral *et al* (2022) reported the results from a single-center prospective study conducted in Kolkata, India, from April 2019 to June 2020. The trial compared the use of thymoglobulin (3 mg/kg) and Grafalon (6 mg/kg) as induction agents in 62 ABO-compatible renal transplant recipients.

Study Design

- The demographic data are presented in Table 5
- Treatment arm: Induction immunosuppressive therapy (methylprednisolone and Grafalon) + standard triple immunosuppression

Table 5: Demographic profile of patients in both groups at the time of the start of the study

Parameters	Thymoglobulin	Grafalon	P value
HD vintage (mo)	11.42	9.23 (± 4.64)	.17
Age (y)	45.39 (± 13.17)	50.45 (± 10.77)	.079
Weight (kg)	55.19 (± 3.95)	58.97 (± 3.85)	<.001
M:F ratio	22:9	25:6	.37
NKD (%)			
ADPKD (%)	1 (3.23)	1 (3.23)	-
CIN (%)	0	1 (3.23)	.014
DN (%)	9 (29)	19 (61)	-
IgAN (%)	3 (9.7)	0	-
Unknown	18 (58)	10 (32)	-
TLC (cells/ μ L)	6.22 (± 1.45)	6.05 (± 1.08)	.86
Hemoglobin (g/dL)	8.72 (± 1.14)	8.57 (± 0.91)	.783
Platelet count (cells/ μ L)	246.77 (± 61.6)	225.84 (± 51.49)	.21
Bilirubin (mg/dL)	0.99 (± 0.13)	2.17 (± 3.06)	.077
SGOT (U/L)	33.97 (± 7.03)	36.42 (± 7.75)	.168
SGPT (U/L)	35.03 (± 7.54)	32.87 (± 7.32)	.183
Dose (mg/kg)	3.01 (± 0.08)	6.72 (± 0.44)	<.00

ADPKD, Autosomal dominant polycystic kidney disease; CIN, Contrast-induced nephropathy; DN, Diabetic nephropathy; HD, Hemodialysis; IgAN, IgA nephropathy; M:F, Male: Female ratio; NKD, Native kidney disease; SGOT, Serum glutamic oxaloacetic transaminase; SGPT, Serum glutamic pyruvic transaminase; TLC, Total leukocyte count.

62 ABO- compatible renal transplant patients (18–65 years) were divided into two groups

One group received 3 mg/kg of thymoglobulin divided for 2 days
The other received 6 mg/kg of Grafalon divided for 2 days

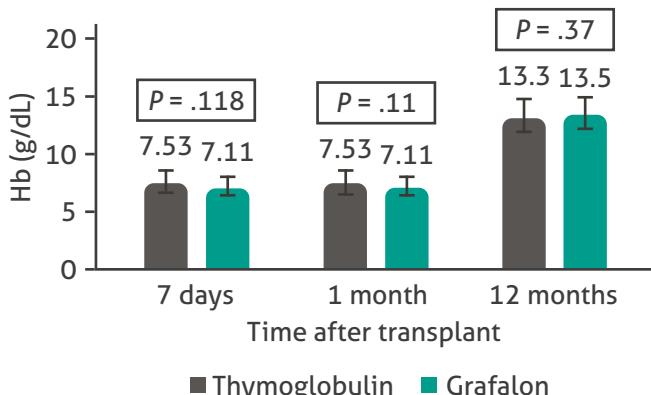
Patients were followed up for 12 months

The two groups were compared for infections, graft function, incidence of rejections, and graft and patient survival

Maintenance immunosuppression included prednisolone 20 mg/d, which was reduced to 5 mg/d by 6 months after transplant and continued; tacrolimus (0.15 mg/kg/d); and mycophenolate sodium (360 mg/three times a day)

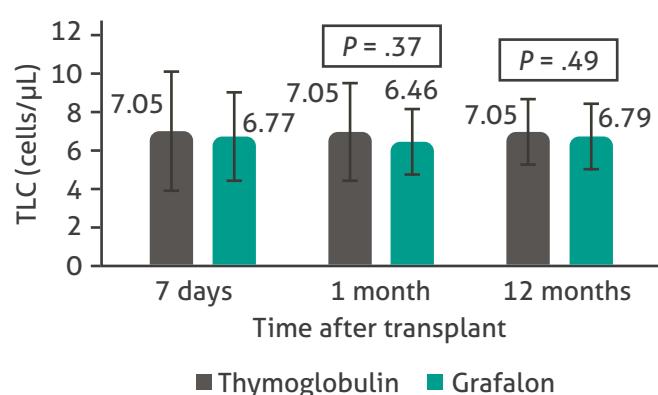
Efficacy and Safety Results

- The hematologic parameters were similar in both the groups at 7 days, 1 month, and 6 months of follow-up; however, at the 12-month follow-up, the lymphocyte count was significantly lower in the thymoglobulin group (Figure 3 [a-h]).
- A significant difference in the delayed graft function was also not observed in both the groups.



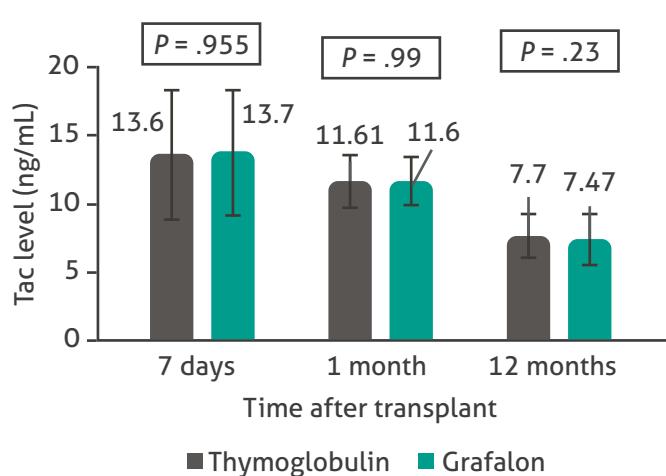
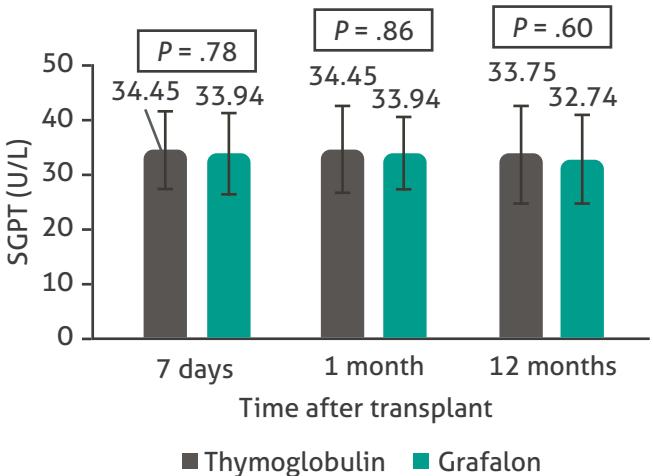
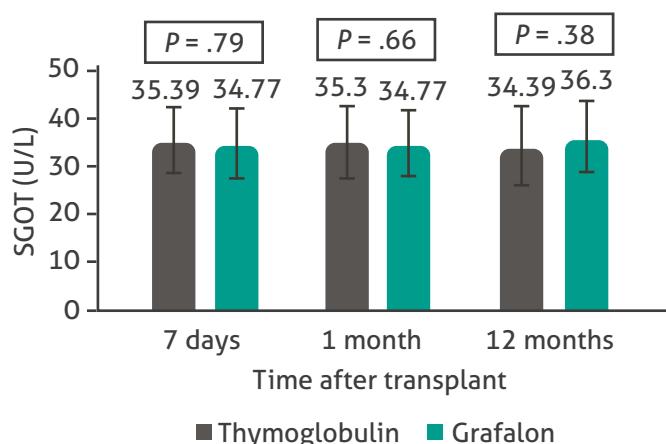
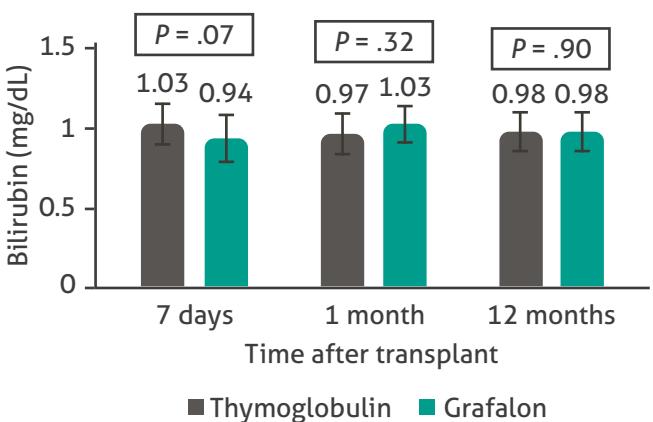
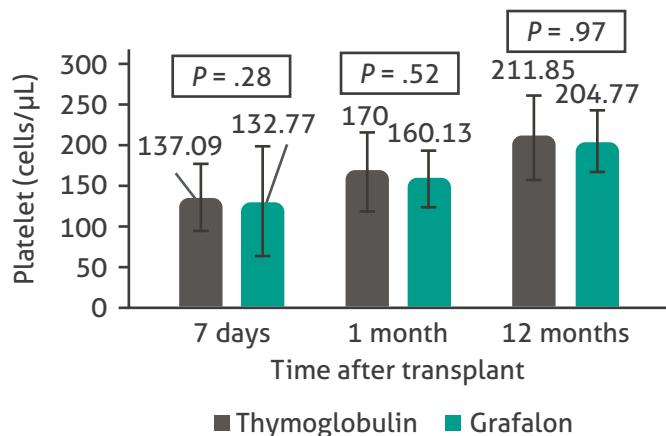
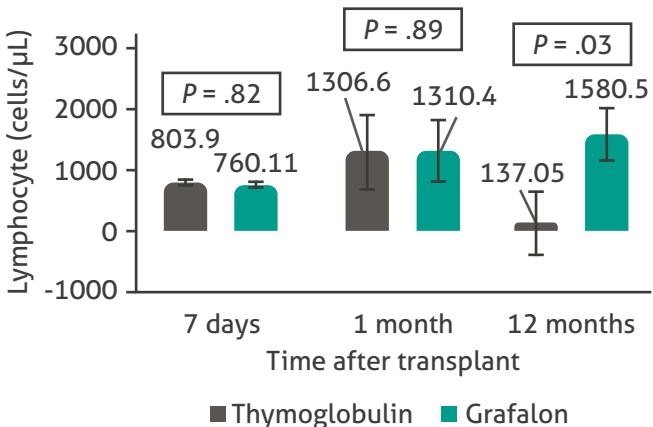
Hb, Hemoglobin.

Figure 3 (a): Hb (g/dL) observed at 7 days, 1 month and 12 months post-transplant



TLC, Total leukocyte count.

Figure 3 (b): TLC (cells/µL) observed at 7 days, 1 month and 12 months post-transplant



SGPT, Serum glutamic pyruvic transaminase.

Figure 3 (g): SGPT (U/L) observed at 7 days, 1 month and 12 months post-transplant

Tac, Tacrolimus.

Figure 3 (h): Tac level (ng/mL) observed at 7 days, 1 month and 12 months post-transplant

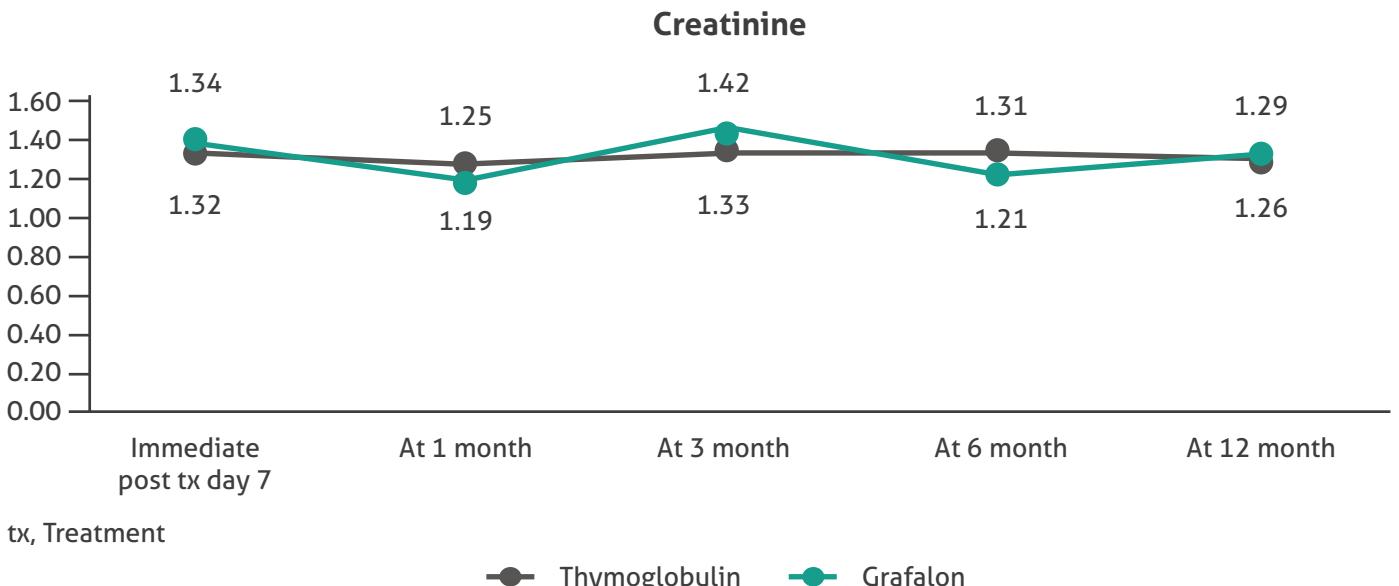


Figure 4: Serum creatinine levels during different stages of follow-up

- The serum creatinine level was similar in both groups on day 7 and at 1, 3, 6, and 12 months (Figure 4).
- Infections requiring hospitalization occurred in 22.58% of recipients in the thymoglobulin group and 19.35% of recipients in the Grafalon group. However, this was not a significant occurrence.
- The various infections that occurred during the follow-up have been summarized in Table 6.
- Sudden cardiac death occurred in a patient from the thymoglobulin group. In the immediate posttransplant period (8-10 days), 5 patients showed rejection episodes, 2 rejections in the thymoglobulin group (6.45%) and 3 in the Grafalon group (9.67%).

Table 6: Infections that occurred in thymoglobulin and Grafalon groups during follow-up

Post-transplant	Thymoglobulin (%)	Grafalon (%)	Total (%)	P value
1 week				
<i>Acinetobacter</i> sepsis	1 (3.22)	1 (3.22)	2 (3.22)	1
CRBSI	1 (3.23)	0	1 (1.61)	.309
LRTI	2 (6.45)	2 (6.45)	4 (6.45)	1
1–3 months				
<i>Escherichia coli</i> sepsis	1 (3.22)	1 (3.22)	2 (3.22)	1
<i>Pseudomonas</i> sepsis	0	1 (3.22)	1 (1.61)	.309
3–6 months				
Pulmonary Kochs	1 (3.22)	0	1 (1.61)	.309
6–12 months				
UTI	1 (3.22)	0	1 (1.61)	.309
COVID-19	0	1 (3.22)	1 (1.61)	.309
Total infections	7 (22.58)	6 (19.35)	13 (21.31)	.755

COVID-19, Coronavirus disease 2019; CRBSI, Catheter-related bloodstream infection; LRTI, Lower respiratory tract infection; UTI, Urinary tract infection.

Key Outcomes

- No significant difference in infective episodes, graft function, the incidence of rejections, posttransplant diabetes mellitus, graft, and patient survival was observed in both groups.
- The efficacy and safety of both thymoglobulin and Grafalon were equivalent in the short term as no difference in graft survival, patient survival, rejection, and infection was observed.

4. Comparison of efficacy and safety between rabbit anti-thymocyte globulin and anti-T-lymphocyte globulin in kidney only transplantation: A retrospective, observational study¹

Kumar *et al* (2022) reported the results of a retrospective single-center study that compared the safety and efficacy of the use of rabbit ATG and Grafalon (ATLG) in kidney-only transplantation. The study was conducted among 127 recipients from January 2014 to June 2019.

Study Design

- The demographic data are presented in Table 7
- Treatment arm: ATLG + standard triple immunosuppression

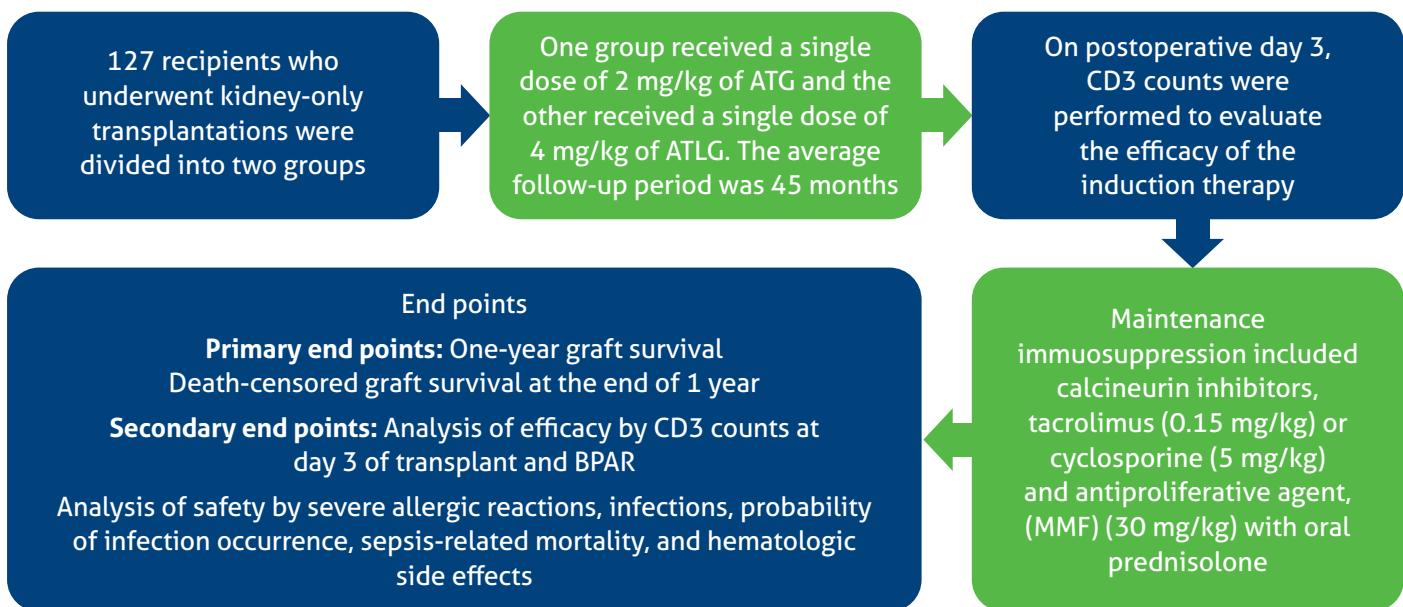


Table 7: Demographic profile of patients in both groups at the time of the start of the study

	ATG	ATLG	P value
n	58	69	
Recipient age (y) ^a	40.8 ± 11.10	42.9 ± 12.30	.082
Donor age (y) ^a	44.8 ± 10.2	44.8 ± 10.2	.988
Recipient gender: Male/female ^b	34 (58)/24 (42)	38 (63.3)/31 (44)	.110
Donor gender: Male/female ^b	19 (32)/39 (68)	27 (33)/46 (67)	.11
Weight (kg) ^a	60.4 ± 14.52	61.7 ± 11.65	.11
Allograft type ^b			
• Live donor	22 (38)	45 (64)	<.001
• Deceased donor	36 (62)	24 (36)	<.001
• ABOI	8 (13.79)	9 (13.04)	.828
• HLA mismatch ^c	6 (0–10)	5 (2–10)	.614
Baseline maintenance steroid (mg) ^b			
• Prednisolone 2.5	28 (48.27)	40 (57.97)	<.05
• Prednisolone 5.0	30 (51.72)	29 (42.02)	.335
Follow-up (months) ^c	45 (12–60)	22 (12–36)	

ABOI, ABO-incompatible; ATG, Antithymocyte globulin; ATLG, Anti-T-lymphocyte globulin; HLA, Human leukocyte antigen; SD, Standard deviation.

^aMean ± SD; ^bn (%); ^cMedian (minimum-maximum).



Efficacy and Safety Results

- The 1-year graft survival rate was 92.7% in the ATLG group and was 87.5% in the ATG group ($P = .04$; Table 8).

- A similar death-censored graft survival rate of 99% was observed in both groups ($P = .258$).
- The single-point mean CD3 level was 211.3 ± 243.8 versus 163.2 ± 156.0 on the third day and was statistically insignificant ($P = .328$).
- A significantly higher rate of bacterial infections (43% vs 30%; $P = .02$) and sepsis-related mortality (11.54% vs 4.34%; $P = .02$) was observed in the ATG group (Table 8).
- An increased probability of occurrence of infection was observed in the ATG group (41.1% vs 27.6%; $P = .03$).

Table 8: Efficacy and safety as determined by patient and graft outcomes

	ATG (58)		ATLG (69)	
	Donor status			
	Deceased, n (%)	Live, n (%)	Deceased, n (%)	Live, n (%)
Bacterial infections ($P = .02$)	16 (44)	9 (40)	11 (45)	9 (22)
	25 (43)		21 (30)	
Fungal infections ($P = .02$)	4 (18)	2 (5)	3 (6)	1 (4)
	6 (10.3)		4 (6)	
CMV infection ($P = .165$)	1 (1.72)		1 (1.44)	
BKV infection	Nil		Nil	
Probability of infection occurrence (%) ($P = .03$)	41.1		27.6	
Sepsis-related mortality ($P = .02$)	4 (11)	2 (9)	2 (8)	1 (2)
	6 (11.54)		3 (4.34)	
Biopsy-proven acute rejection episodes ($P = .128$)	13 (36)	6 (27)	8 (33)	12 (26)
	19 (32)		20 (29)	
Biopsy-proven acute rejection episodes (ABMR) in ABOI ($P = .34$)	-	1 (12)	-	1 (13)
Mean time for rejection after transplant (days) ($P = .237$)	54 ± 30		59 ± 29	
Posttransplant malignancy	Nil		Nil	
1-year graft survival (%) ($P = .04$)	86	89	90	95.4
	87.5		92.7	
Death-censored graft survival at 1 year (%) ($P = .258$)	99		99	
Single-point mean CD3 level on the third day ($P = .328$)	211.3 ± 243.8		163.2 ± 156.0	

ABMR, Antibody-mediated rejection; ABOI, ABO-incompatible; ATG, Anti-thymocyte globulin; ATLG, Anti-T-lymphocyte globulin; BKV, BK virus; CD, Cluster of differentiation; CMV, Cytomegalovirus.

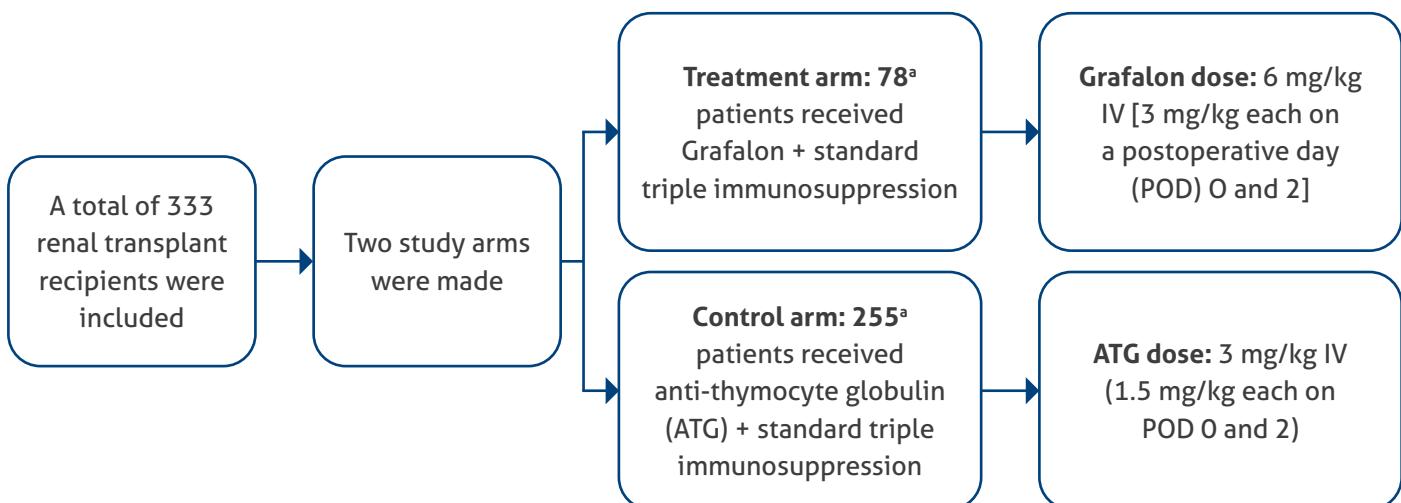
Key Outcomes

- The 1-year graft survival was better in the ATLG (Grafalon) group compared with the ATG (thymoglobulin) group.
- Grafalon was associated with a decreased rate of bacterial infections as well as sepsis-related mortality when used as an induction agent.

5. Grafalon vs thymoglobulin as an induction agent in renal transplantation – A retrospective study³

Jha *et al* (2021) conducted a single-center retrospective study to assess the outcome of Grafalon and compare it with thymoglobulin as an induction agent in patients with renal transplants from January 2017 to October 2019. In this study, 333 renal transplant recipients were included.

Study Design



^aIt was a retrospective study with skewed randomization of the patient population where the control (thymoglobulin) arm had more than thrice the patients in the treatment (Grafalon) arm, which was a major limitation of the study.

Efficacy and Safety Results

- Patient survival and death-censored graft survival were comparable between the two groups (Table 9).
- The rate of BPAR was significantly higher in the Grafalon group (12.8%) than in the thymoglobulin group (5.1%), $P = .04$.

Table 9: Patient outcomes

Variables	Grafalon (n = 78)	Thymoglobulin (n = 255)	P value
Patient survival	99%	98.8%	1
Death-censored graft survival	99%	100%	.23
eGFR (on the last follow-up) (mL/min)	73.1 ± 20.5	75.9 ± 23.8	.35
BPAR	12.8% (n = 10)	5.1% (n = 13)	.04 ^a
Infections	12.8% (n = 10)	20.7% (n = 53)	.13
CMV infection	0	1% (n = 2)	1
BKV infection	2.5% (n = 2)	0.4% (n = 1)	.14
Post-transplant malignancy	0	0	1
NODAT	5.1% (n = 4)	6.7% (n = 17)	.79

BPAR, Biopsy-proven acute rejection; CMV, Cytomegalovirus; eGFR, Estimated glomerular filtration rate; NODAT, New-onset diabetes after transplant.

^aSignificant.

- The patient survival in the Grafalon group and the thymoglobulin group was 99% and 98.8%, respectively ($P = 1.0$; Figure 5).
- The death-censored graft survival was comparable between the two groups (99% in Grafalon vs 100% in thymoglobulin; $P = .23$; Figure 6).
- BPAR was reported more significantly in the Grafalon group (12.8% vs 5.1%; $P = .04$; Figure 7).

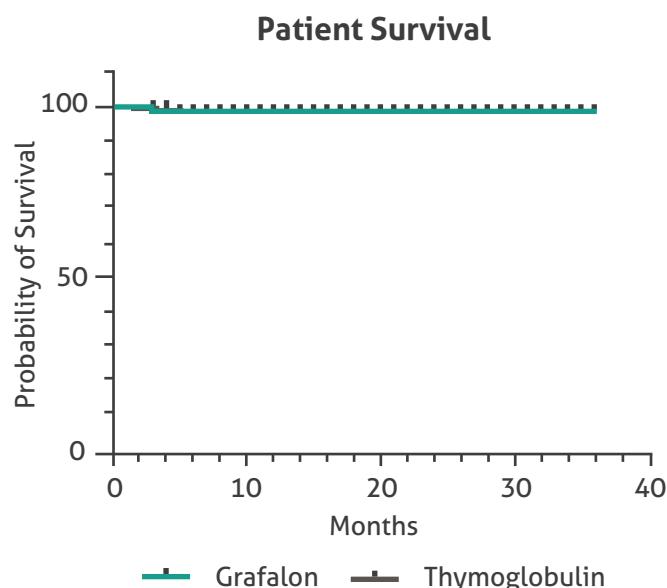


Figure 5: Kaplan–Meier graph comparing the patient survival between the Grafalon and thymoglobulin groups

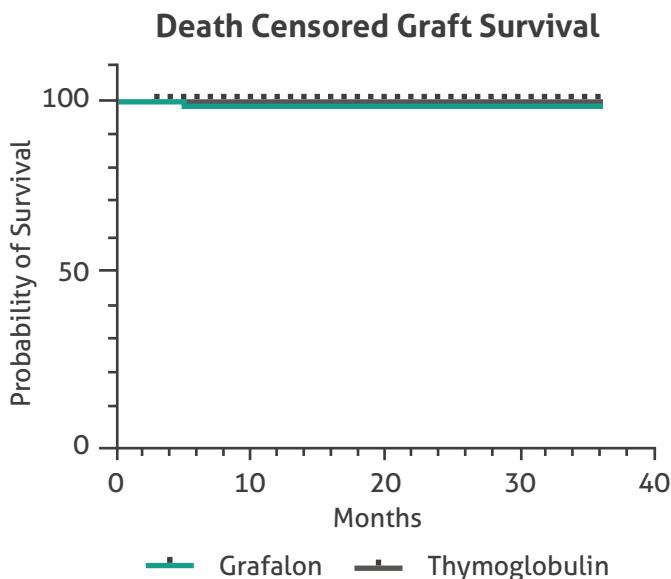


Figure 6: Kaplan–Meier graph comparing the death-censored graft survival between the Grafalon and thymoglobulin groups

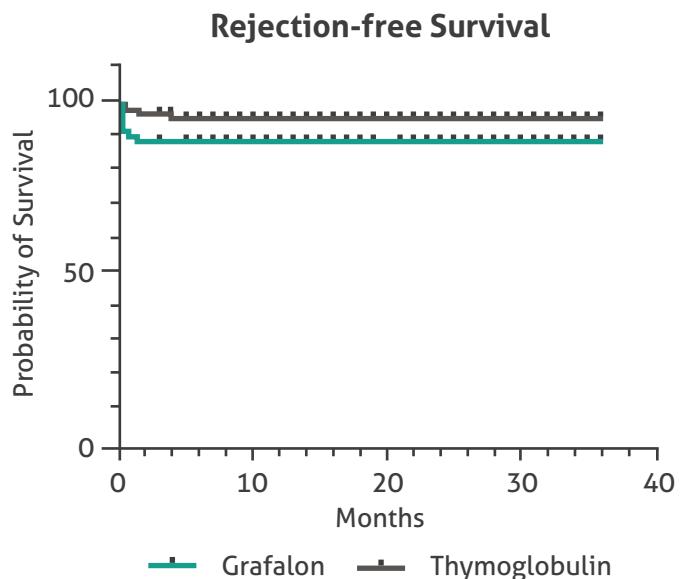


Figure 7: Kaplan–Meier graph comparing the acute rejection-free survival between the Grafalon and thymoglobulin groups

Key Outcome

- When used as an induction agent, Grafalon (6 mg/kg dose) was related to a significantly higher rate of BPARs compared with that of thymoglobulin (3 mg/kg dose), although with a similar short-term patient and death-censored graft survival, graft function, and infection rates.

6. Retrospective study of ATLG/Anti-T-lymphocyte immune globulin (Grafalon) vs basiliximab (Simulect) as an induction agent for kidney transplantation – A single center experience⁹

Namagondlu G *et al* (2019) conducted a retrospective study and analyzed data from kidney transplant recipients who received either Grafalon or Simulect as induction agent from January 2011 to August 2018.

Study Design

All study patients underwent living donor kidney transplantation

Grafalon group (*n* = 23)

Mean age: 41.5 years (range: 19-64)
Mean Tacrolimus dose for the first
3 months: 10.9 ng/mL (3.8-30)



Grafalon 3-5 mg/kg body weight IV was given at the time of induction

Simulect group (*n* = 12)

Mean age: 48 years (range: 27-70)
Mean Tacrolimus dose for the first
3 months: 11.6 ng/mL (.7-22)



Two doses of Simulect 20 mg IV was given as per standard protocol at induction and 4 days post-transplant

Both groups received IV Methyl prednisolone at the time of induction

Standard maintenance immunosuppression: Tacrolimus (0.1 mg/kg/day), Mycophenolate mofetil 500 mg or mycophenolate sodium 720 mg twice daily, and Oral prednisolone 20 mg once daily

Target trough tacrolimus levels 3 months post-transplant: 8-10 ng/mL

Renal functions were evaluated

Table 10: Renal function in Grafalon and Simulect groups

	Grafalon group (<i>n</i> = 23)	Simulect (<i>n</i> = 12)
Mean CIT	45.6 mins (35-60 mins)	49.4 mins (35-90 mins)
1 st transplant	20 (85%)	11 (91.6%)
2 nd transplant	3 (15%)	1 (8.3%)
Serum creatinine (mg/dL) at 3 months	1.25 (.5-2.5)	1.2 (.9-1.8)
eGFR (mL/min) at 3 months	74	71
Serum creatinine at 6 months	1.37 (.7-2.4)	1.37 (.8-3)
eGFR at 6 months	72	66.2

CIT, Cold ischemic time; eGFR, Estimated glomerular filtration rate.



Table 11: Complications observed in less than 12 months

	Grafalon group (n = 23)	Simulect (n = 12)
Rejection episodes	4 (17.3%)	2 (16.6%)
Cell mediated	3	2
Antibody mediated	1	0
Infections	3	3
Surgical complications	2	1
Other (MI)	0	1
Graft loss	0	0
Mortality	0	0

MI, Myocardial infarction.

- At one year of kidney transplantation, all grafts in both groups were functional, with no mortality.

Key Outcomes

- There is no significant difference between the two groups in terms of 6 months renal function and 1 year graft, patient survival or complication rates.
- In low-risk kidney transplants, Grafalon is a less expensive and non-inferior option to Simulect.

7. Prospective randomized trial to evaluate the efficacy of single low dose ATG induction in renal transplant recipient with spousal kidney¹⁰

Kumar A *et al* (2002) reported the results from a prospective randomized trial that evaluated the efficacy of single-dose (3.5–5 mg/kg) ATG in 30 patients (30 patients were taken as control) between July 1996 and January 2000. A standard triple drug immunosuppression was administered in both the groups with an additional single shot of Grafalon (ATLG) in the study group.

Study Design

The demographic data are presented in Table 12.

In both groups, all the patients were administered with standard triple drug immunosuppression including prednisolone, azathioprine, and cyclosporine

Peroperatively recipients received 500 mg of hydrocortisone before releasing the vascular clamp

An additional dose of 200 mg of ATLG in 200 mL of saline was also given to patients in the study group. This infusion was initiated at induction of anesthesia and ended before revascularization of the graft

At the last follow-up, 26 patients (86.3%) in the induction group and 24 patients (80%) in the control group were examined for graft function, rejection, episodes, anti-rejection therapy, infections, and development of malignancy

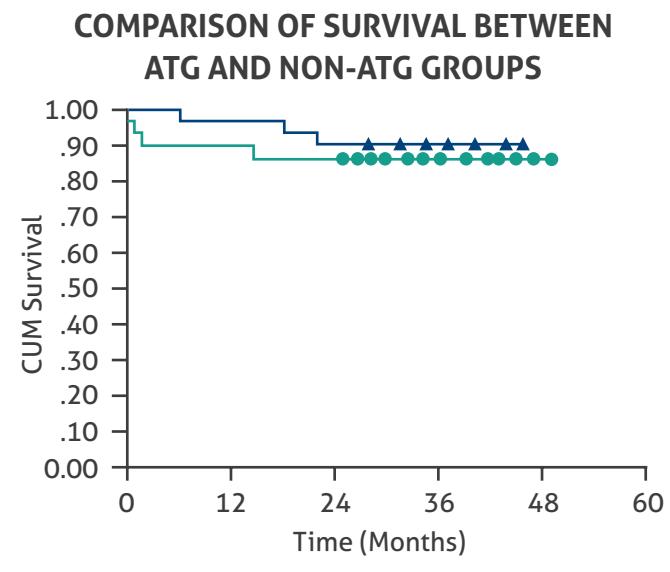
Table 12: Demographic data

	Study	Control
No. of patients	30	30
Age (years)	27–57	20–62
Mean ± SD	44.26 ± 9.49	41.26 ± 10.05
Sex (M:F)	24:6 (4:1)	26:4 (6.5:1)
Follow-up (months)	25–46	24–48
Mean ± SD	24.46 ± 9.26	24.28 ± 9.66

F, Female; M, Male; SD, Standard deviation.

Efficacy and Safety Results

- At the last follow-up, the patient and graft survival were 86.3% and 83.3% in the study group, respectively, and 80% and 70% in control group, respectively (Figure 8).
- On the other hand, impaired graft function (serum creatinine >2 mg%) was not observed in induction group and was observed in 10% in control group (Table 13).
- Acute rejection episodes were more in the control group (43.3%) as compared with the induction group (23.3%).



ATG, Antithymocyte globulin.

Figure 8: Kaplan-Meier chart showing comparison of survival between ATG (study) and non-ATG (control) groups

Table 13: Patient and graft survival at follow-up

	Study	Control	P value
Patient survival	26	24	.244
Stable graft function	25	21	.111
Impaired graft function	-	3	-
Patient expired	2	4	.194
Graft loss	1	-	-
Lost to follow-up ^a	2	2	.500

^aPatients lost to follow-up were presumed to be dead.

- There were six deaths (two patients in study group; four patients in control group) due to medical problems. Also, in both the groups, two patients were lost to follow-up (Table 13). In induction group, one patient required graft nephrectomy, because of severe graft dysfunction with significant proteinuria.
- The incidence of UTI was observed more in the study group compared with the control group (46.7% vs 23.3%); however, the incidence of other sites infections was comparable in both the groups. Pulmonary tuberculosis was observed in two patients in each group (Table 14).

Table 14: Infections

	Study	Control	P value
UTI	14	7	.029
Pneumonitis	5	6	.369
Fungal	5	6	.369
Herpes zoster	4	5	.358
Pulmonary tuberculosis	2	2	.500
Diabetic foot	1	-	-
Bone tuberculosis	1	-	-

UTI, Urinary tract infection.

Key Outcomes

- Single, low-dose ATLG does not show any improvement in graft survival at 2.5 years.
- Single, low-dose ATLG reduces graft rejection episodes; however, it is associated with higher urinary infection rates. A long-term follow-up is needed to see any benefit of less rejection on long-term graft survival.

Pooled Analysis of Six Studies

Methods:

Data is analyzed using R software version 4.2.1 and Microsoft Excel. Chi-square test/ Fisher's Exact test is used to check the association of categorical variables. A *P* value less than or equal to 0.05 indicates statistical significance.

Note: The count of event is obtained by rounding p^*n to the nearest integer where p is the percentage and n is the total number of subjects in the group.

Results:

Pooled data of different outcomes in the Grafalon arm from various studies

Table 15: Pooled analysis of patient survival, death-censored graft survival, infection, pooled analysis of different types of infections, graft rejection and impact of dose on graft rejection in the Grafalon arm

	Yes (%)	No (%)	P value
Patient survival	713 (95.8)	31 (4.2)	-
Death-censored graft survival ^a	706 (98.9)	8 (1.1)	-
Infection			
Bacterial	160 (24.8)	584 (75.2)	Graft rejection ^c
Fungal	137 (17.8)	-	4 mg/kg
Viral	9 (1.5)	-	6 mg/kg
CMV	6 (0.8)	-	
BKV	3 (0.4)	-	
VZV	5 (0.6)	-	
Graft rejection ^c	65 (9.8)	601 (90.2)	-
4 mg/kg	27 (27.3)	72 (72.7)	
6 mg/kg	38 (6.7)	529 (93.3)	<0.0001*

^aKumar A *et al.* study is excluded as no death-censored graft survival data is available

^bBacterial infection (UTI) is the most common type of infection

^cJha *et al* study was excluded due to skewed data as a result of incomparable sample size

*From Chi-square test, it was observed that the association of graft rejection was significantly different between the two dosage groups (*P* <0.05).

Pooled data of different outcomes from 3 studies comparing Grafalon and thymoglobulin – Kumar SS et al, Thukral et al and Jha et al.

Table 16: Association of patient survival, death-censored graft survival, total infection, types of infections, graft rejection in thymoglobulin and Grafalon groups

Group	Grafalon	Thymoglobulin	P value
Patient survival			0.4533*
Survived (%)	170 (95.5)	333 (96.8)	
Died (%)	8 (4.5)	11 (3.2)	
Death-censored graft survival			0.2691 ^f
Survived (%)	176 (98.9)	343 (99.7)	
Died (%)	2 (1.1)	1 (0.3)	
Infection			0.3564*
Yes (%)	41 (23.0)	92 (26.7)	
No (%)	137 (76.9)	252 (73.2)	
Type of infection			
Bacterial	36 (20.2)	85 (24.7)	0.4188 ^f
Fungal	4 (2.24)	6 (1.74)	0.4618 ^f
Viral			
CMV	1 (0.5)	3 (0.4)	0.5789 ^f
BKV	2 (1.1)	1 (0.3)	0.2691 ^f
Others	1 (0.5)	0	-
Graft rejection ^a			0.9229*
Yes (%)	23 (23.0)	21 (23.6)	
No (%)	77 (77.0)	68 (76.4)	
Dose effect on graft rejection ^a	Yes (%)	No (%)	
Grafalon 4 mg/kg	20 (29.0)	49 (71.0)	
Grafalon 6 mg/kg	3 (9.7)	28 (90.3)	
Thymoglobulin 2 mg/kg			0.0406 ^f
Thymoglobulin 3 mg/kg			0.0076 ^f
Yes (%)	19 (32.8)	39 (67.2)	
No (%)	2 (6.5)	29 (93.5)	

*From Chi square test, it was observed that the association of patient survival and total infection were not significantly different between the two groups ($P>0.05$).

^fFrom Fisher's exact test, it was observed that the association of death-censored graft survival and type of infection were not significantly different between the two groups ($P>0.05$).

^aJha et al study was excluded due to skewed data as a result of incomparable sample size.

^fFrom Fisher's exact test, it was observed that the association of dose effect on graft rejection was significantly different between the two groups ($P<0.05$).

Inference:

- Out of 744 patients on Grafalon, 713 patients survived (95.8%), infections were seen in 160 patients (24.8%) and graft rejection was seen in 65 patients (9.8%) (Table 15).
- With the available data, death-censored graft survival could be analyzed in 714 patients on Grafalon, of which graft survival was seen in 706 patients (98.9%) (Table 15).
- Pooled analysis with the available data showed bacterial infection (17.8%) as the most common type of infections, while fungal infections (1.5%) was infrequent, and serious viral infections like CMV, BKV and VZV were very rare (<1% each) (Table 15).
- There were significantly lesser graft rejections seen in patients receiving Grafalon 6 mg/kg compared to those receiving Grafalon 4 mg/kg (Table 15).
- There was no significant difference in patient survival, death-censored graft survival or graft rejection between thymoglobulin and Grafalon groups (Table 16).
- There was a higher percentage of total infection rate seen in thymoglobulin group (26.7%) than Grafalon group (23.0%), though the difference was not statistically significant (Table 16).
- There were no significant differences in different infection types between thymoglobulin and Grafalon groups, however there were numerically more bacterial infections with thymoglobulin (24.7%) compared to Grafalon (20.2%) (Table 16).
- In the studies comparing Grafalon with thymoglobulin, significant difference on graft rejection with different doses was observed. Grafalon showed significantly lower rate of graft rejection with 6 mg/kg dose compared to 4 mg/kg. Similarly thymoglobulin showed significantly lower rate of graft rejection with 3 mg/kg dose compared to 2 mg/kg (Table 16).

Executive Summary of Key Insights from the Analysis

01

What should be the ideal dose of Grafalon in the Indian population?

From the pooled analysis, a dose of 6 mg/kg of Grafalon has been found more effective in preventing graft rejections, and can be considered the optimal dose in patients with low to intermediate immunological risk. However, in high immunological risk patients, a higher dose might be warranted and further studies are needed to determine the optimal dose.

Studies have shown good clinical outcomes in Indian transplant patients with individualized dosing approach.⁷

02

What was the effect of different doses of Grafalon on graft rejection?

Based on the findings of the pooled analysis, the risk of graft rejection was lower among patients receiving 6 mg/kg Grafalon compared to those receiving 4 mg/kg.

03

Compare the effect of Grafalon with thymoglobulin on patient survival and death-censored graft survival. What is the long-term impact of Grafalon on patient survival and death-censored graft survival?

Based on the pooled analysis, no significant difference was seen in patient survival and death-censored graft survival between Grafalon and thymoglobulin.

Results from different studies have shown similar patient survival and death-censored graft survival even beyond 1 year. Moreover, even after 5 years of kidney transplant, patients who had steroid avoidance continued to show similar efficacy and safety with Grafalon compared to those who received steroids for the initial 6 months.^{4, 11}

04

How effective is Grafalon compared with thymoglobulin as an induction agent in the case of renal transplantation?

Pooled analysis of studies in India shows that Grafalon at a dose of 6 mg/kg is not inferior to ATG in prevention of AR in low to intermediate risk kidney transplant recipients with lesser rate of bacterial infections with Grafalon than with thymoglobulin.¹ Hence Grafalon can be as effective and safer than thymoglobulin in Indian patients undergoing a kidney transplant.

05

What is the role of Grafalon in lymphocyte, CD4, and CD8 T-cell recovery?

A study has reported that a single Grafalon dose led to quicker lymphocyte, CD4, and CD8 T-cell recovery along with comparatively less long-term immune-inhibitory potential.¹

06

What are the adverse reactions associated with Grafalon?

Some of the adverse reactions associated with Grafalon include thrombocytopenia, anemia, leukopenia, tachycardia, vomiting, nausea, diarrhea, and abdominal pain, among others.²

07

What are the contraindications for Grafalon use?

Grafalon is contraindicated in the following conditions: (i) hypersensitivity to the active substance or any other excipient; (ii) patients with untreated bacterial, viral, parasitic, or mycotic infections; (iii) solid organ transplant patients with severe thrombocytopenia (<50,000 platelets/ μ L); and (iv) patients with malignant tumors except for cases in which stem cell transplantation is performed as a part of the treatment.²

08

What are the common infections seen in patients on Grafalon?

Patients on Grafalon were found to develop bacterial, viral, and fungal infections. Bacterial infection was seen in 17.8% of the patients. Among those, UTI, LRTI, bacterial pneumonia, bacterial sepsis and tuberculosis were common, UTI being the most common infection.^{1,3,4,7,8,10} CMV (0.8%), BKV (0.4%) and VZV (0.6%) infections were seen very rarely.^{1,3,4} Fungal infections (1.5%) included candidiasis and fungal pneumonia.^{7,8}

09

Compare the incidence of infections between Grafalon and thymoglobulin

Based on the studies, following are the infections seen with Grafalon compared to thymoglobulin:

- From the pooled analysis there was no significant difference seen any of the infections between the two groups, though numerically **more bacterial infections were seen with thymoglobulin (24.7%) vs Grafalon (20.2%)**.
- However, in Thukral *et al* study, overall **rate of infection was higher in thymoglobulin group (22.58%) vs Grafalon group (19.35%)**.⁸
- In Kumar SS *et al* study, bacterial infection rate was **significantly higher in thymoglobulin group (43%) vs Grafalon (30%) ($P = 0.02$)**.¹

10

Is there any risk of developing post-transplant malignancy after being treated with Grafalon in kidney transplant patients?

No, there is not any risk of developing posttransplant malignancy in kidney transplant patients as observed in various studies, although the follow-up duration was short.³

11

Is ALC suppression expected with Grafalon?

Yes, ALC suppression is expected when treating patients with Grafalon. If ALC is already <200 cells/ μ l, an additional dose of Grafalon is not recommended until the counts are >200 again.⁴

12

What is the effect of Grafalon compared with thymoglobulin on graft and patient survival in patients undergoing renal transplantation?

Thymoglobulin and Grafalon were found to be similar regarding safety and efficacy without any statistically significant difference in terms of rejections, infections, graft survival and patient survival in patients undergoing renal transplantation.⁸

13

What is the effect of Grafalon on patient survival or complication rates compared with basiliximab (Simulect) after 1 year of use?

A study showed that there is no significant difference in 1-year graft survival, patient survival, 6-months renal function or complication rates between the groups that received either Grafalon or Simulect.⁹

14

Does ABO incompatibility influence graft survival and episodes of infections in patients with kidney transplantation?

The ABO incompatibility did not have any significant effect on the Grafalon and thymoglobulin groups in respect to graft survival and infections.^{1,3}

Future directions:

Evidence from real-world practice on the effectiveness and safety of Grafalon has shown that a personalized dosing approach based on CD count resulted in better clinical outcomes in living-donor transplant patients with moderate immunological risk. Hence it is imperative to have a tailor-made benefit versus risk assessment done to individualize the regimen, prevent rejections, and minimize the risk of infections in these patients.

Further studies on Grafalon and its comparison with other formulations with larger sample sizes, randomized, blinded, and prospective analysis longer follow-up is required to assess the other differences. Prospective trials are also required along with more pieces of evidence in high-risk and ABO-incompatible kidney transplant patients.

Abbreviations

ABMR, Antibody-mediated rejection; ABOI, ABO-incompatible; ACR, Acute cellular rejection; AR, Acute rejection; ALC, Absolute lymphocyte count; ATG, Antithymocyte globulin; ATG-F, Antithymocyte globulin-Fresenius; ATLG, Anti-human T-lymphocyte globulin; BKV, BK virus; BPAR, Biopsy-proven acute rejection; CD, Cluster of differentiation; CMV, Cytomegalovirus; ESKD, End-stage kidney disease; HBV, Hepatitis B virus; HCV, Hepatitis C virus; HIV, Human immunodeficiency virus; HLA, Human leukocyte antigen; IV, Intravenous; KT, Kidney transplantation; LRTI, lower respiratory tract infections; MMF, Mycophenolate mofetil; POD, postoperative day; UTI, Urinary tract infection; VZV, Varicella-zoster virus.

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