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A 3-month, Multicenter, Randomized, Open-label Study to Evaluate the Impact on Wound Healing of the Early (vs Delayed) Introduction of Everolimus in De Novo Kidney Transplant Recipients, With a Follow-up Evaluation at 12 Months After Transplant (NEVERWOUND Study)

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Background. The risk of wound healing complications (WHCs) and the early use of mammalian target of rapamycin inhibitors after kidney transplantation (KT) have not been fully addressed. **Methods.** The NEVERWOUND study is a 3-month, multicenter, randomized, open-label study designed to evaluate whether a delayed (ie, 28 ± 4 d posttransplant) immunosuppression regimen based on everolimus (EVR) reduces the risk of WHC versus EVR started immediately after KT. Secondary endpoints were treatment failure (biopsy-proven acute rejection, graft loss, or death), delayed graft function, patient and graft survival rates, and renal function. **Results.** Overall, 394 KT recipients were randomized to receive immediate (N = 197) or delayed (N = 197) EVR after KT. At 3 months, WHC-free rates in the immediate EVR versus delayed EVR arm, considering the worst- and best-case scenario approach, were 0.68 (95% confidence interval [CI], 0.62-0.75) versus 0.62 (95% CI, 0.55-0.68) (log-rank *P* = 0.56) and 0.70 (95% CI, 0.64-0.77) versus 0.72 (95% CI, 0.65-0.78) (log-rank *P* = 0.77), respectively. The 3- and 12-month treatment failure rates, delayed graft function and renal function, and patient and graft survival were not different between the arms. **Conclusions.** The early introduction of EVR after KT did not increase the risk of WHC, showing good efficacy and safety profile.

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INTRODUCTION

In kidney transplantation (KT), the introduction of new immunosuppressive agents may offer the opportunity to reduce adverse events (AEs) and personalize the therapy, while maintaining a good feasibility and efficacy. The

introduction of mycophenolic acid (MPA) and mammalian target of rapamycin inhibitors (mTORis) led, in selected patients, to the reduction or elimination of calcineurin inhibitors (CNIs) in early post-KT.¹⁻⁵ Everolimus (EVR) (Certican; Novartis Pharma AG, Basel, Switzerland) is a

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mTORi immunosuppressant drug with antiproliferative properties that reduces growth factor-stimulated lymphocyte proliferation.^{6,7} In the experimental model and KT human trials, EVR showed good safety and efficacy with an acceptable tolerability,⁸⁻¹¹ while reducing vascular smooth muscle cells proliferation⁶ and neointimal growth¹²⁻¹⁵ and leading to a reduction in graft arteriosclerosis.¹⁶

Several studies suggested that Sirolimus, an mTORi drug, was associated with an increased rate of wound healing complications (WHCs) after solid organ transplantation,¹⁷⁻²³ an effect potentially related to the inhibition of the activation and proliferation of fibroblast cells. On the contrary, even if the evidence is still scarce, EVR did not show inferiority versus other immunosuppression (IS)^{24,25} and only the drug exposure seems to be related with WHC, especially in severely obese recipients.^{26,27}

In the CALLISTO study,²⁸ a 12-month randomized multicenter trial, KT patients at risk of delayed graft function (DGF) were randomized to receive EVR either immediately after KT or in a delayed setting. Composite endpoints were DGF, biopsy-proven acute rejection (BPAR), death, and WHC. The authors eventually specify that the immediate introduction of EVR was not associated with any disadvantage in terms of graft recovery or wound healing.

Considering the small population (ie, 139 randomized patients) of the CALLISTO study and the sample size estimation (calculated for the composite endpoint)—as well as the lack of any randomized trial on mTORi fitted for WHC only—the present study was designed with the aim of evaluating whether the delayed administration of EVR (ie, 28 ± 4 d posttransplant) reduces the risk of WHC in de novo KT recipients.

MATERIALS AND METHODS

Study Design

The NEVERWOUND study was a 3-month, multi-center, randomized, prospective, open-label study with an observational follow-up of 12 months, conducted in 22 Italian kidney transplant centers from November 2011 to December 2015 with the goal of evaluating whether a delayed (ie, 28 ± 4 d posttransplant) EVR-based IS regimen reduces the risk of WHC versus EVR started immediately after KT.

During the screening (day -2 to day 0), patients were assessed for eligibility for the study. At day 0, all patients underwent KT and started the induction treatment as per clinical practice. In all cases, the transplantation consisted in the standard pelvic operation, with heterotopic extraperitoneal placement of the graft and ureteroneocystostomy according to Lich et al²⁹ and Gregoir.³⁰ At baseline (day 1 to day 2), transplanted patients eligible for the study were randomized (ratio, 1:1) to 1 of the 2 following treatment arms: immediate EVR (IE) or delayed EVR (DE) (Figure 1) via a Web-based system and stratified by age at transplant (≤ 60 or >60) and pretransplant diabetes mellitus status.³¹

Upon completion of the 3-month treatment period (CORE phase study), patients entered a 9-month observational follow-up period, where they were treated as per local clinical practice. During the follow-up visit performed 12 months ($-1/+6$ mo) after KT, information was collected on patient and graft survival, renal function, acute rejection, malignancies, wound assessment, new-onset diabetes mellitus, serum creatinine, and blood levels of EVR and cyclosporine A (CsA).

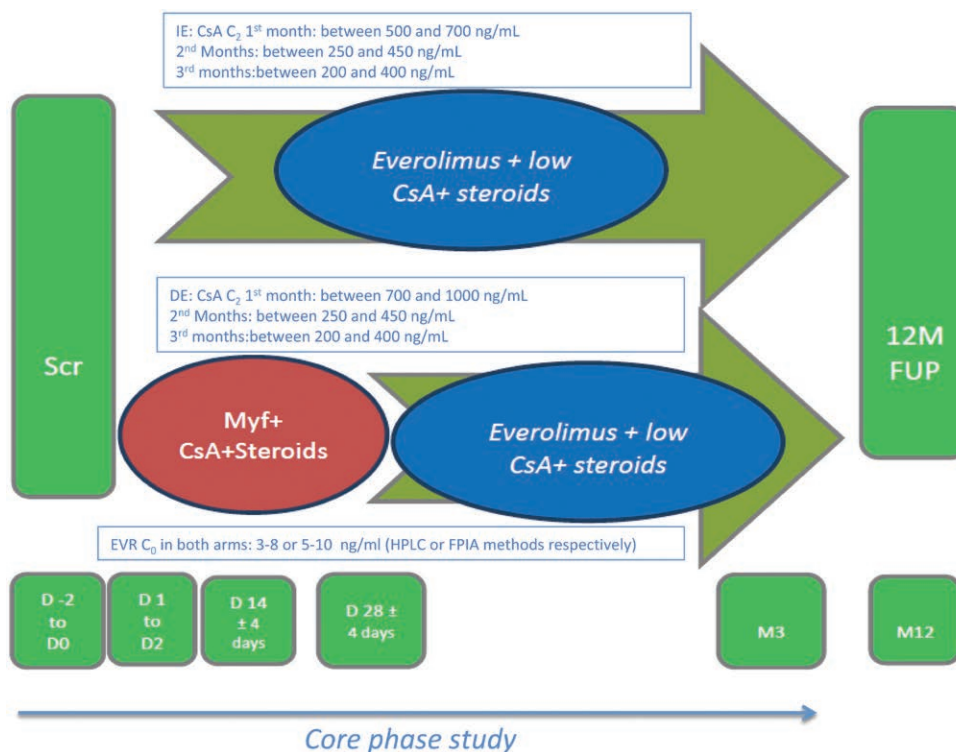


FIGURE 1. Study design. C₀, immunosuppression blood levels, before morning dose; C₂, immunosuppression blood levels, 2h after morning dose; CsA, cyclosporine A; D, day; DE, delayed everolimus; EVR, everolimus; FPIA, fluorescence polarization immunoassay; FUP, follow-up; HPLC, high-performance liquid chromatography; IE, immediate everolimus; M, month; Myf, myfortic; scr, screening.

The study complied with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice and the Declaration of Helsinki and its amendments. A written informed consent was obtained from all patients, and each center's ethical committee approved the study.

Treatment Regimens

In the IE, EVR was started within 48 hours after graft reperfusion, at the starting dose of 0.75 mg BID, adjusted to target EVR trough level at 0 hour (immunosuppression blood levels, before morning dose [C_0]) to reach a range of 3–8 ng/mL (using the high-performance liquid chromatography method) or 5–10 ng/mL (using the fluorescence polarization immunoassay method); CsA (Neoral; Novartis Pharma AG, Basel, Switzerland) was administered BID per os within 48 hours of graft reperfusion, at a starting dose of 4 mg/kg/d. The dose was then adjusted to reach and maintain CsA immunosuppression blood levels 2 h after morning dose (C_2) between 500 and 700 ng/mL. When the EVR target level was reached, CsA was tapered up to reach C_2 levels between 250 and 450 ng/mL during month 2 and between 200 and 400 ng/mL up to month 3.

In the DE, MPA (Myfortic; Novartis Pharma AG, Basel, Switzerland) was administered within 48 hours after graft reperfusion, at the dose of 720 mg BID. At 28 ± 4 days after KT, MPA was discontinued and EVR was started at 0.75 mg BID to reach and maintain C_0 3–8 ng/mL (high-performance liquid chromatography method) or 5–10 ng/mL (fluorescence polarization immunoassay method). CsA was administered BID per os within 48 hours of graft reperfusion, at a starting dose of 6–8 mg/kg/d. The dose was adjusted to reach C_2 levels of 700–1000 ng/mL until day 28 ± 4 . The dose was then tapered up to reach the same IE arm C_2 levels.

Intravenous prednisone (or equivalent) was administered perioperatively, and oral corticosteroids were started the day after, at 20 mg/d, and then tapered according to the center's clinical practice. Induction therapy was administered, as per local clinical practice (namely IL2 receptor antibodies [Ab-IL2-R] or antithymocyte globuline [ATG]).

Inclusion criteria for the study were as follows: all adult (>18 y) male or female patients who had received first or second single KT from deceased donor or living related were eligible for the study.

Patients were excluded in the following cases: recipients of multiple organ transplants, including 2 kidneys; women of childbearing potential, unless they were using 2 birth control methods; pregnant or nursing (lactating) women; recipients with historical or current peak panel reactive antibodies >50% or unacceptable human leukocyte antigen(s), platelets <75000/mm³, absolute neutrophil count <1500/mm³, leukocytes <2500/mm³, or hemoglobin <7 g/dL; symptoms of significant somatic or mental illness; history of malignancies within the past 5 years; human immunodeficiency virus (HIV) positivity; evidence of severe liver disease; severe active infections; evidence of drug or alcohol abuse; or body mass index (BMI) >30 kg/m².

Primary Endpoint

The primary objective of the study was to evaluate—during the treatment period (90 ± 4 postoperative days)—whether the delayed administration of EVR in de novo KT

recipients reduced the risk of WHC versus the immediate administration.

WHCs were defined as fluid collection, including hematoma and lymphocele (defined as serum >5 cm fluid collections evaluated by ultrasound [assessed at 14 ± 4 , 28 ± 4 , 90 ± 4 , and 360 ± 30 postoperative days]), requiring or not intervention; prolonged lymphatic drainage (ie, lymphorrhea) >150 mL/24 h at 7 postoperative days; wound dehiscence, wound infection, and incisional hernia (evaluated after 3 and 12 mo).

Secondary Endpoints and AEs

The secondary objectives were as follows: treatment failure rate (composite endpoint: BPAR [graded according to Banff 97 criteria], graft loss, death); death-censored graft survival rate; graft survival rate; patient survival rate; incidence and duration (days) of DGF (arbitrarily defined as the need of dialysis in the first week after transplant); renal function, through estimated glomerular filtration rate (estimated glomerular filtration rate—modification of diet in renal diseases formula); proteinuria (>1000 mg/24 h or >1.0 protein/creatinine in a spot urine sample); safety; and tolerability (incidence of AEs, serious AEs, infections).

Sample Size

A 2-group continuity-corrected chi-square test with a 0.05 2-sided significance level had a 90% power to detect the difference between a group IE percentage of 0.65 and DE percentage of 0.80 (odds ratio, 2.15) when the sample size in each arm was 198. Three hundred ninety-six patients were therefore enrolled.

Study Population

The following populations were defined in the study:

Intent-to-treat population consisting in all the randomized patients who were treated.

Safety population consisting in all the randomized patients who were treated and had ≥ 1 safety assessment.

Statistical Analysis

Continuous data are summarized by mean, SD, median, first and third quartile, and minimum and maximum. Categorical data are presented by absolute and relative frequencies (n and %) or contingency tables and, where relevant, with their 95% confidence interval (CI) limits.

The presence/absence of ≥ 1 WHC was analyzed also by means of a logistic regression model, considering as covariates the type of induction (basiliximab vs ATG), capacity of the center (>10 vs ≤ 10 patients), diabetes mellitus (yes vs no), age at transplantation, and BMI (<25 vs ≥ 25 kg/m²).

The primary analysis was performed on the safety population.

The time to event was analyzed by means of a Cox model, considering the same covariate used in the logistic regression model. The hazard ratio (HR) with a 95% CI was presented. Kaplan-Meier curves of time were also calculated. Changes in renal function were analyzed by the ANCOVA model, considering the same covariate used in the logistic regression model.

For the comparison of proportion, the chi-square test or the Fisher exact test was used, while the Wilcoxon test was used for continuous nonnormally distributed data.

All the analysis were produced using 9.4 SAS for Windows.

In order to manage the missing data, patients who discontinued the study before reaching the endpoint, those who discontinued EVR for any reason, and those with missing information about WHC were considered failures, according to the *worst-case scenario approach*, and successes, according to the *best-case scenario approach* (Sensitivity Analysis).

RESULTS

Study Population

Three hundred ninety-nine patients were screened; of these, 394 were randomized (IE = 197; DE = 197), and 383 (98.2%) received ≥ 1 dose of the study drug and were included in both the intent-to-treat and the safety populations. Two patients were treated differently than the assigned treatment arm; 336 patients out of 383 (87.7%) completed the 3-month study (IE = 181; DE = 155); of these, 310 (80.9%) (IE = 161 [81.7%]; DE = 149 [75.6%]) completed the CORE study on treatment. In particular, 12 patients in the IE arm discontinued the study due to graft loss ($n = 4$); consent withdrawn ($n = 3$); death ($n = 2$); administrative reasons ($n = 2$); and lost to follow-up ($n = 1$). In the DE arm, 35 patients discontinued due to protocol violation (total $n = 28$; due to a nonswitch to

EVR for unknown reason $n = 25$; clinical reasons $n = 2$; error $n = 1$); death ($n = 3$); consent withdrawn ($n = 2$); graft loss ($n = 1$); or administrative reasons ($n = 1$). In the IE arm, 32 patients (16.2%) discontinued EVR at 3 months, mainly due to AEs ($n = 24$, 12.4%), while in the DE arm, only 12 patients (7.4%) required EVR discontinuation. Three hundred seven patients had their 12-month follow-up visit (IE = 159 [82.4%]; DE = 148 [77.9%]) (Figure 2).

Demographics on recipients' and donors' baseline characteristics of the 2 treatment arms are detailed in Table 1.

In the DE arm, the switch to EVR was successful in 161 (84.7%) (median time to switch, 29 d [range, 24–62 d]).

At the end of CORE study, the majority of patients in the IE arms were compliant with the C₀-EVR protocol (namely 76.2%, 80.4%, and 79.4% after 14 \pm 4 and 28 \pm 4 days and 3 mo, respectively), while 10% showed blood levels <3–8 ng/mL and 10% above such levels. In the DE arm, 91.8% of patients had levels within range, while in 8.2% of them, levels were below 3–8 ng/mL.

Primary Endpoint: WHCs

Considering the worst-case scenario, the 3-month rate of patients without WHC in the IE and DE arms was 0.68 (95% CI, 0.62-0.75) versus 0.62 (95% CI, 0.55-0.68) (log-rank test: $P = 0.56$) (Figure 3). This accounted for a 16% increase in the relative risk for WHC occurrence in the DE arm (HR, 1.16; 95% CI, 0.81-1.64; $P = 0.42$). The results were confirmed considering the best-case scenario (Figure 4); drop out patients seem to have a different impact on the results, according to the above scenarios,

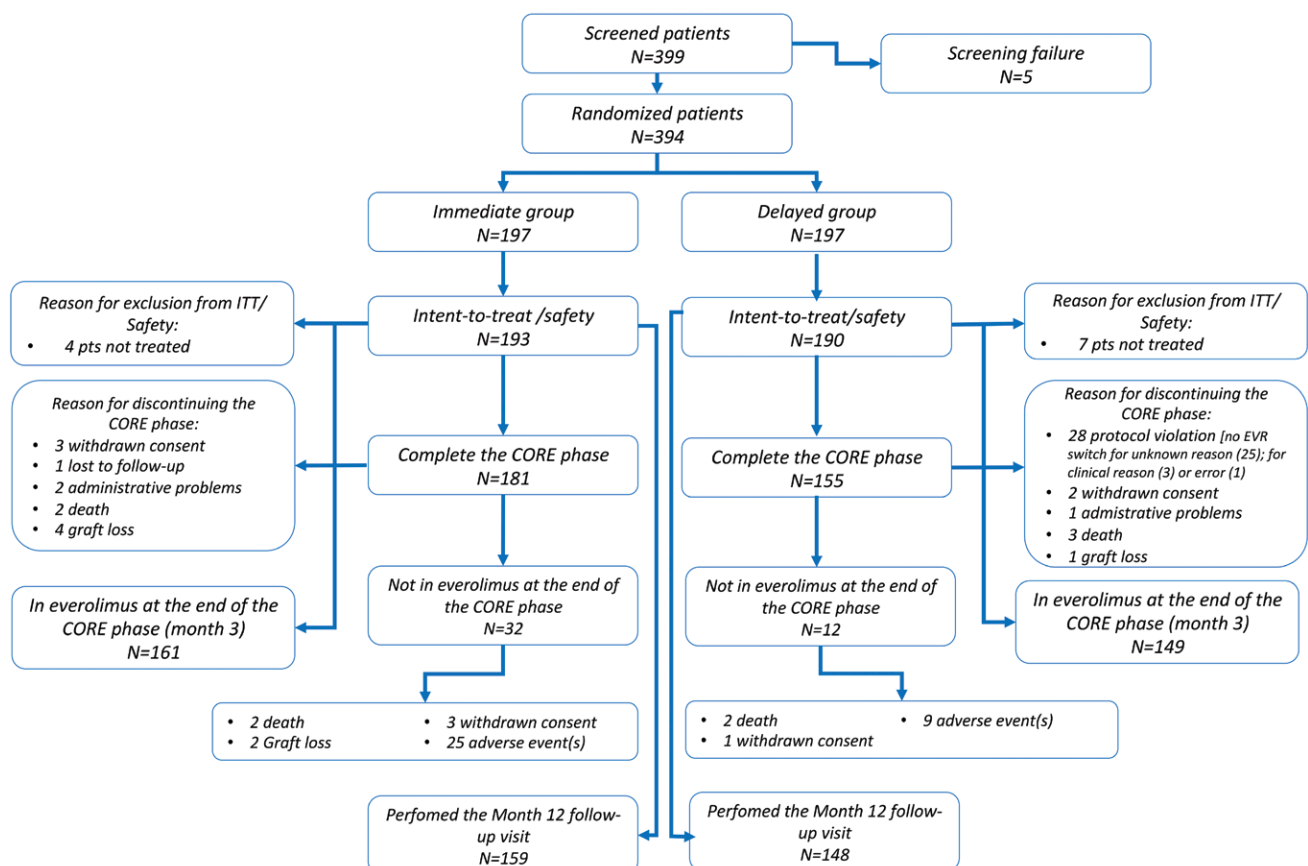


FIGURE 2. Patient disposition. EVR, everolimus; ITT, intent-to-treat; pts, patients.

TABLE 1.
Demographics and baseline recipient and donor characteristics (safety population)

Recipient	IE N = 193	DE N = 190
Age (y) at screening, mean (SD)	51.46 (11.37)	51.19 (12.29)
Male (%)	134 (69.4)	132 (69.5)
Presence of diabetes mellitus (%)	17 (8.8)	17 (9.0)
BMI ^a , mean (SD)	24.65 (3.28)	24.41 (3.29)
BMI ^a ≥25 (%)	89 (46.6)	86 (46.5)
End-stage disease leading to KT (%)		
Glomerulonephritis/glomerular disease	56 (29.0)	57 (30.0)
Polycystic disease	45 (23.3)	40 (21.0)
Hypertension/nephrosclerosis	32 (16.6)	17 (9.0)
Pyelonephritis	5 (2.6)	3 (1.6)
Diabetes mellitus	5 (2.6)	5 (2.6)
Unknown origin	21 (10.9)	38 (20.0)
Other	29 (15.0)	30 (17.8)
PRA (%), mean (SD)	1.33 (6.57)	2.46 (8.13)
Cold ischemia time (h), mean (SD) ^b	12.31 (4.90)	12.39 (5.50)
Induction therapy (%)		
Basiliximab	152 (78.8)	157 (82.6)
ATG	37 (19.2)	31 (16.3)
None	4 (2.1)	2 (1.0)
Donor		
Age (y), mean (SD)	52.77 (15.34)	53.21 (14.39)
Male (%)	100 (51.8)	94 (49.5)
Living donor (%)		
Related	5 (2.6)	6 (3.2)
Unrelated	3 (1.5)	5 (2.6)

^aIE: N = 191; DE: N = 185.

^bCold ischemia time considered only in deceased donor.

ATG, antithymocyte globuline; BMI, body mass index; DE, delayed everolimus; IE, immediate everolimus; KT, kidney transplantation; SD, standard deviation; PRA, panel reactive antibodies.

especially in the DE arm, where 12.6% did not switch to EVR and therefore dropped out of the study (Table 2).

The most frequent complications were as follows: fluid collection (IE = 32 [16.6%] vs DE = 33 [17.4%]; *P* = 0.84) and lymphocele (IE = 20 [10.4%] vs DE = 30 [15.8%]; *P* = 0.12). A lower percentage of patients experienced lymphorrhea (IE = 5 [2.59%] vs DE = 13 [6.84%]; *P* = 0.05) and hematoma (IE = 14 [7.25%] vs DE = 5 [2.63%]; *P* = 0.04) (Table 3).

Similar results were observed over the entire follow-up period (12 mo), considering the worst- (Figure 5) and best-case (Figure 6) scenario (Table 2).

After 12 months, the DE arm showed a greater incidence of lymphocele versus the IE arm (IE = 21 [10.88%] vs DE = 35 [18.42%]; *P* = 0.04); conversely, the occurrence of hematoma was greater in the IE arm (IE = 15 [7.77%] vs DE = 5 [2.63%]; *P* = 0.02) (Table 3).

Considering the CORE phase and worst-case scenario, the multivariate Cox model identified ATG as a risk factor for the occurrence of WHC (HR, 1.62; 95% CI, 1.06-2.46; *P* = 0.02); however, these findings were not confirmed in the best-case scenario approach (HR, 1.47; 95% CI, 0.93-2.33; *P* = 0.10). BMI <25 kg/m² was identified as a predictor of WHC-free status at 12 months, both in the worst (HR, 0.66; 95% CI, 0.47-0.92; *P* = 0.02) and best-case scenario (HR, 0.64; 95% CI, 0.44-0.93; *P* = 0.02) (Table 4).

Secondary Efficacy Endpoints

At 3 months, considering the worst-case scenario, the composite efficacy treatment failure (ie, death/graft loss/BPAR) was 22 in the IE arm (11.4%) versus 40 (21.0%) in the DE arm (log-rank: *P* = 0.06; HR, 1.54; 95% CI, 0.90-2.64; *P* = 0.11). Fitting the best-case scenario approach, the treatment failure rate was lower in both arms (IE = 16 [8.3%] vs DE = 13 [6.8%]; log-rank: *P* = 0.57; HR, 0.63;

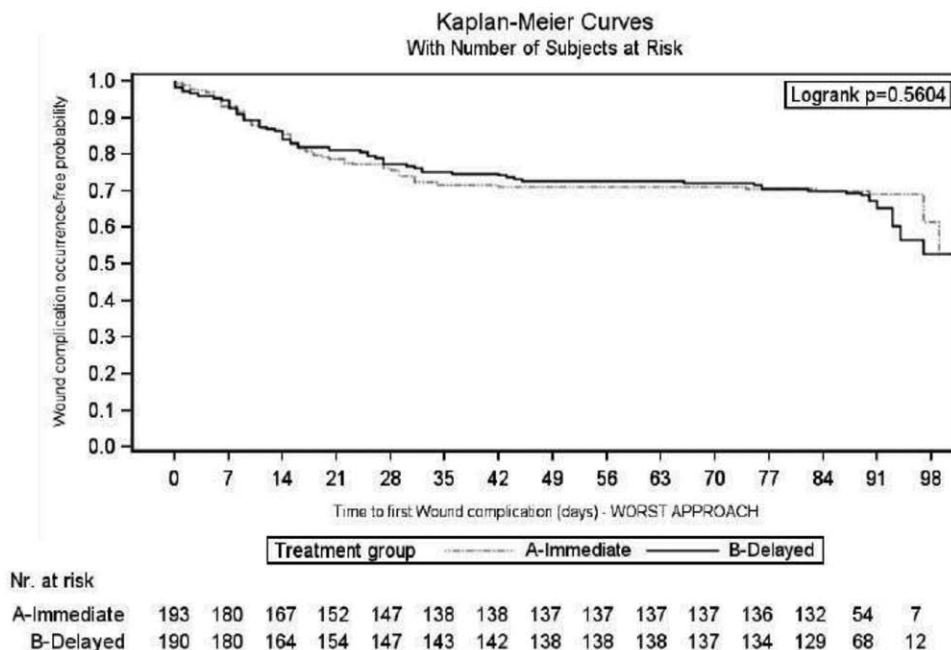


FIGURE 3. Kaplan-Meier curve of time to first wound healing complication during the first 3 mo after transplantation: worst-case scenario approach (intent-to-treat population). Nr. at Risk, number at risk.

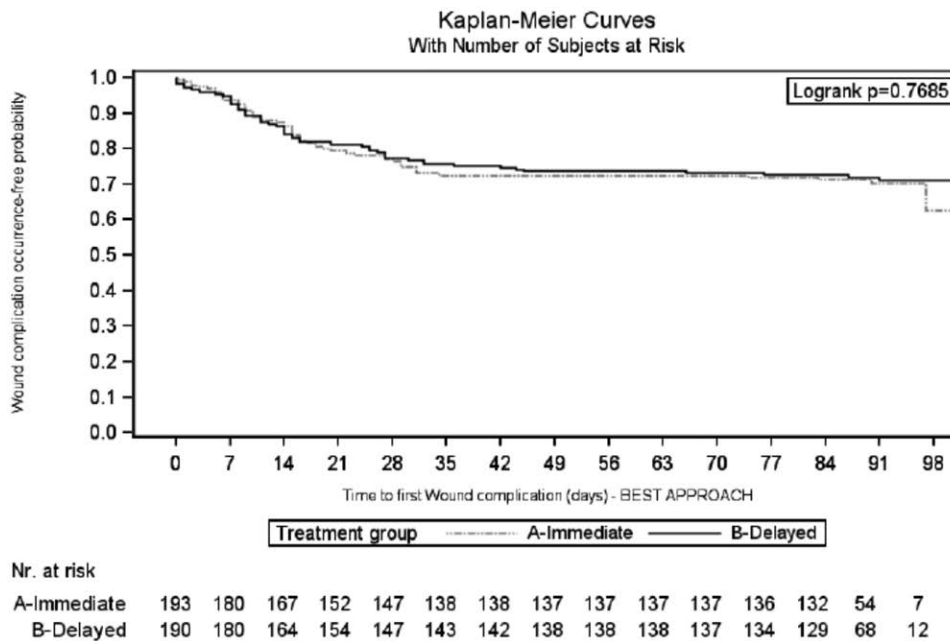


FIGURE 4. Kaplan-Meier curve of time to first wound healing complication during the first 3 mo after transplantation: best-case scenario approach (intent-to-treat population).

TABLE 2.

WHC at 3 and 12 mo: ITT population

	IE N = 193		DE N = 190		Log-rank	Cox proportional hazard model: DE vs IE	
	N (%)	95% CI	N (%)	95% CI		HR (95% CI)	P
3 mo: worst case							
Patients without WHC	132 (68.4)	62-75	117 (61.6)	55-68	0.5604	1.16 (0.81-1.64)	0.4156
3 mo: best case							
Patients without WHC	136 (70.5)	64-77	136 (71.6)	65-78	0.7685	0.98 (0.67-1.43)	0.8997
12 mo: worst case							
Patients without WHC	127 (65.8)	59-72	113 (59.5)	52-66	0.3252	1.21 (0.86-1.69)	0.2757
12 mo: best case							
Patients without WHC	131 (67.9)	61-74	132 (69.5)	63-76	0.7543	0.97 (0.67-1.40)	0.8667

In the Cox proportional hazard model, the following confounding factors were included: age at KT, BMI (<25 vs ≥25), center dimension (>10 vs ≤10), presence of diabetes mellitus, and induction (ATG vs basiliximab).

ATG, antithymocyte globuline; BMI, body mass index; CI, confidence interval; DE, delayed everolimus; HR, hazard ratio; IE, immediate everolimus; ITT, intent-to-treat; KT, kidney transplantation; WHC, wound healing complications.

95% CI, 0.29-1.39; $P = 0.25$). The graft loss in IE was due to acute rejection ($n = 3$); primary nonfunction ($n = 1$); or graft artery thrombosis ($n = 1$), while in DE, it was due to renal vein thrombosis. The difference between the best- and the worst-case scenario approach was due to a difference between the arms in the dropout rate, since in the DE arm, 12% (24/190) of patients did not switch to EVR. Similar results were observed considering the 12-month follow-up period (Table 5).

The 1-year patient and graft survival rates, in the IE and DE arms, were 99.0% versus 98.4% (log-rank: 0.6565) and 96.9% versus 98.4% (log-rank: 0.3337), respectively (Figures 7 and 8). Five patients died during the CORE study (IE = 2; DE = 3), due to acute myocardial infarction (IE = 1; DE = 1); cardiovascular accident (IE = 1; DE = 1); and cerebral hemorrhage (DE = 1). None of the deaths were considered by the investigators to be EVR related.

Considering the entire study period, no difference was observed either in BPAR (IE = 19 [9.84%] vs DE = 16 [8.42%]; $P = 0.61$) or in DGF (IE = 46 [23.8%] vs DE = 60 [31.6%]; $P = 0.12$) (Table 5).

No differences in renal function and proteinuria were observed at months 3 and 12 (Table 6).

Relevant concomitant medications are listed in Table 7.

Adverse Events

There were no differences between the arms with regard to patients who experienced ≥1 AE/serious AE/infection or in the number of patients who discontinued treatment due to AE/infection (Table 8). Specifically, 171 patients (88.6%) in the IE arm versus 174 (91.6%) in the DE arm experienced ≥1 AE ($P = 0.42$); of these AEs, 71 (36.8%) in the IE arm and 53 (27.9%) in the DE arm were considered EVR related ($P = 0.06$). The shorter exposure to

TABLE 3. Wound healing complication details at 3 and 12 mo: ITT population

	IE	DE	P
	N = 193	N = 190	
	N (%)	N (%)	
Occurred within 3 mo			
Lymphorrhea	5 (2.6)	13 (6.8)	0.0493
Hematoma	14 (7.2)	5 (2.6)	0.0373
Lymphocele	20 (10.4)	30 (15.8)	0.1150
Fluid collection	32 (16.6)	33 (17.4)	0.8372
Wound dehiscence	11 (5.7)	5 (2.6)	0.1335
Wound infections	7 (3.6)	6 (3.2)	0.7999
Incisional hernia	0	3 (1.2)	0.1211
Occurred within 12 mo			
Lymphorrhea	6 (3.1)	13 (6.8)	0.0925
Hematoma	15 (7.8)	5 (2.6)	0.0238
Lymphocele	21 (10.9)	35 (18.4)	0.0368
Fluid collection	34 (17.6)	37 (19.5)	0.6401
Wound dehiscence	12 (6.2)	5 (2.6)	0.0884
Wound infections	7 (3.6)	6 (3.2)	0.7999
Incisional hernia	2 (1.0)	3 (1.6)	0.6834

P values were calculated by means of a chi-square test except for hernia, where the Fisher exact test was used.
DE, delayed everolimus; IE, immediate everolimus; ITT, intent-to-treat.

EVR in the DE arm and the fact that 12% of patients did not switch to EVR may have contributed to this result. Patients who showed ≥1 serious AE/infection in the IE versus DE arm were 73 (37.8%) and 61 (32.1%), respectively (P = 0.29). Patients who permanently or temporarily discontinued EVR due to AEs/infections in the IE versus DE arm were 29 (15.0%) versus 21 (11.1%) (P = 0.32) and 18 (9.3%) versus 23 (12.1%) (P = 0.47), respectively.

Thirty-three patients experienced ≥1 cytomegalovirus infection (IE = 14 [7.2%] and DE = 19 [10%]; P = 0.33). Of them, only 6 recipients (14%) received cytomegalovirus prophylaxis (P = 0.19). Eleven patients experienced new onset of diabetes mellitus (IE = 5 [3.1%] vs DE = 6 [4.1%]; P = 0.76) (Table 8).

Considering the overall cohort, 1 patient (in the DE arm) experienced a new occurrence of renal cancer.

DISCUSSION

The immediate introduction of EVR after KT does not increase the risk of WHC. In fact, in our population, the DE use was not associated with a decreasing risk of WHC versus those who received IE. Both the worst- and best-case scenario of this analysis—which were fitted to avoid any bias due to missing information—clearly showed that no difference in terms of WHC was identified between the arms. The immediate use of EVR in the study period resulted in a WHC-free rate between 68% and 70% (considering the worst- and best-case approach) versus 62% and 72% in those who received a delayed dose; the most common complications in IE were fluid collection (17%), lymphocele (10%), and wound dehiscence (6%), which were not different at 3 months from the DE arm. Interestingly, at 12 months, the DE arm showed a higher incidence of lymphocele, while hematoma seemed to be more frequent in the IE arm. The risk of WHC increased by >60% in case of use of ATG induction, confirming what already stated by Pourmand et al³² in 2012, suggesting that Ab-IL2-R (namely Basiliximab) should be an induction of choice in mTORi-based IS (also in the delayed introduction protocol). The risk also increases in obese recipients (BMI, >30 kg/m²)²⁷; in fact, in our series, BMI <25 kg/m² proved to have a protective role on WHC; therefore, we suggest to avoid a combination therapy of ATG and mTORi, especially in overweight recipients to decrease the “synergistic effects” on WHC.

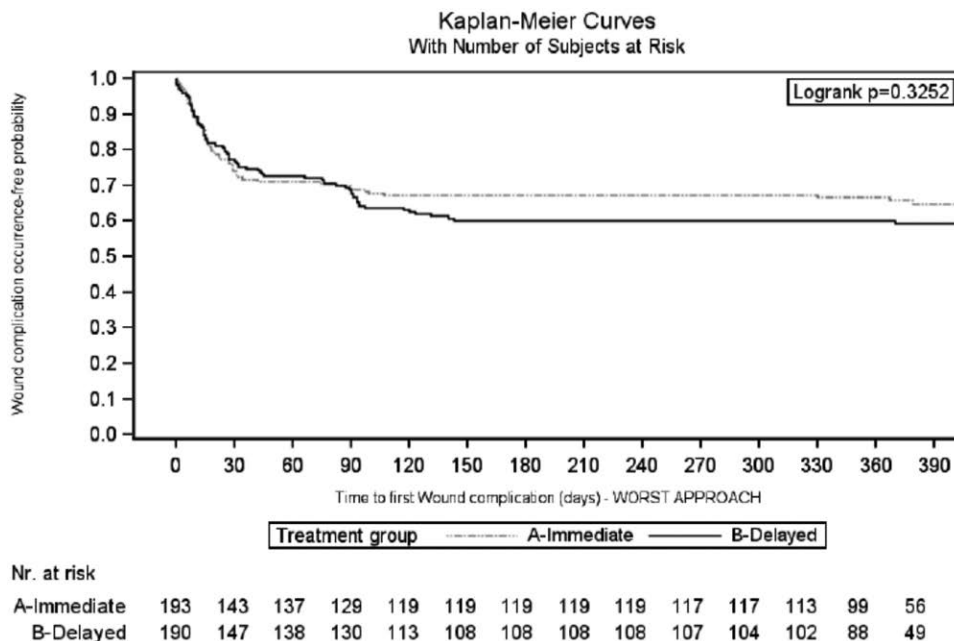


FIGURE 5. Kaplan-Meier curve of time to first wound healing complication during the 12 mo after transplantation: worst-case scenario approach (intent-to-treat population).

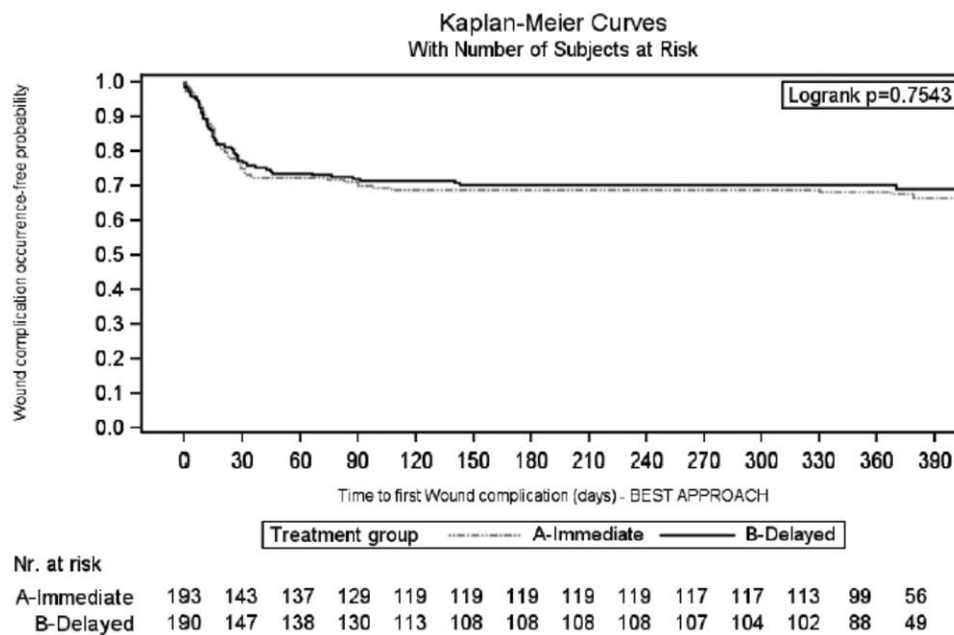


FIGURE 6. Kaplan-Meier curve of time to first wound healing complication during the 12 mo after transplantation: best-case scenario approach (intent-to-treat population).

Even if no robust data had previously showed the clear role of mTORi on WHC in the KT setting, these results seem to be consistent with the preliminary issues and previously mentioned CALLISTO study, in which no difference was observed in the incidence or severity of WHC in KT recipients receiving either micophenolate mofetil (MMF) or EVR as de novo immunosuppressive drug.²⁸

These findings, on the contrary, differ from the studies on sirolimus-based IS, since sirolimus seems to lead to WHC following KT^{17,19-22}; the intriguing question thus arises as to why a difference may potentially exist between each other. Pharmacokinetic variations (higher bioavailability and shorter half-life of EVR)³³⁻³⁵ or the loading doses and high exposure level of sirolimus immediately after KT may partly contribute to explaining the difference between the drugs. The high sirolimus exposure (namely C_0 , 8–30 ng/mL) in the first clinical experiences and association with MPA in CNI-free protocol immediately after transplantation can explain the unfavorable effect on wound healing.²⁶

In 2013, Cooper et al³⁶ showed that the higher blood level of EVR (namely >8 ng/mL) was also associated with an increasing risk (HR, 1.69; 95% CI, 1.20-2.38; $P = 0.002$) of WHC and the initial dose of 1.5 mg seems to be more protective than 3 mg with regard to WHC. Even if the present study has not explored the role of EVR blood levels on the incidence of WHC (only 10% of patients in the IE arm were above 3–8 ng/mL), we are strongly convinced that a dose adjustment aimed at maintaining a level between 3 and 8 ng/mL is fundamental to decrease or avoid the WHC.

The role of EVR on wound healing was also investigated in heart transplantation by the so-called EVERHEART study. Similar in study design to the present trial, the study showed no superiority in terms of WHC in the DE arm; however, similarly to CALLISTO, the study was not powered for WHC only, but for

composite end-points (ie, wound healing delays, pericardial and pleural effusion, and renal insufficiency). Therefore, it was not fit to investigate the final role of EVR in wound healing.³³

In the study period, treatment failure does not significantly differ between the arms, but—when considering the entire follow-up period (12 mo) and worst-case scenario—the immediate introduction of EVR seems to be superior, mostly in the graft loss and death rates; this results, however, should not be considered consistent, since they were not confirmed in the best-case scenario, so they cannot be considered conclusive. The 12-month BPAR rate in the IE arm was about 10%, therefore over 10% less than in the CALLISTO study; this first of all confirms the high efficacy of the IE regimen, and also indicates that a high CNI-exposure immediately after KT can be safely avoided. This can also be confirmed by the rate of DGF; as a matter of fact, the IE and DE arms showed the same DGF rates (23% vs 30%); these results are completely in accordance with the CALLISTO study (25%) and larger Symphony study,¹⁹ which reported DGF rates ranging between 32% and 36% in CNI-treated recipients. From the current study and CALLISTO series, we can assert—albeit in disagreement with other single experiences^{17,18,34,35}—that the immediate use of mTORi does not influence the initial poor function in KT, a finding that was also confirmed by a similar renal function assessed between the treatment arms throughout the follow-up.

Finally, permanent or temporary discontinuations of EVR were much less than in the CALLISTO study; indeed, in our series, the IE arm permanently discontinued therapy in only 15% of the cases and temporarily in 9%, while in the DE arm, discontinuation occurred in about 12% of the cases. Controversially, the CALLISTO series reported a 25% discontinuation rate; this could be explained—as stated by the authors themselves—by the higher EVR trough levels reached

TABLE 4.**Cox multivariate analysis on the main risk factors influencing the WHC**

Time point	Approach	Factors	HR (95% CI)	β estimate (SE)	P
3 mo	Worst case	Age at transplantation (y)		0.01 (0.01)	0.4674
		BMI			
		<25 vs ≥25 kg/m ²	0.76 (0.53-1.08)		0.1262
		Center size			
		>10 vs ≤10 patients	1.46 (0.78-2.73)		0.2353
		Diabetes mellitus			
		Yes vs no	0.91 (0.48-1.73)		0.7793
		Treatment arm			
12 mo	Worst case	Delayed everolimus vs immediate everolimus	1.16 (0.81-1.64)		0.4156
		Type of induction			
		ATG (antithymocyte globulin) vs basiliximab (Simulect)	1.62 (1.06-2.46)		0.0246
		Age at transplantation (y)		0.00 (0.01)	0.5488
		BMI			
		<25 vs ≥25 kg/m ²	0.66 (0.47-0.92)		0.0159
		Center size			
		>10 vs ≤10 patients	1.40 (0.77-2.53)		0.2720
3 mo	Best case	Diabetes mellitus			
		Yes vs no	0.81 (0.43-1.52)		0.5035
		Treatment arm			
		Delayed everolimus vs immediate everolimus	1.21 (0.86-1.69)		0.2757
		Type of induction			
		ATG (antithymocyte globulin) vs basiliximab (Simulect)	1.47 (0.98-2.20)		0.0653
		Age at transplantation (y)		0.01 (0.01)	0.3828
		BMI			
12 mo	Best case	<25 vs ≥25 kg/m ²	0.74 (0.50-1.08)		0.1214
		Center size			
		>10 vs ≤10 patients	1.42 (0.72-2.82)		0.3154
		Diabetes mellitus			
		Yes vs no	0.93 (0.48-1.82)		0.8312
		Treatment arm			
		Delayed everolimus vs immediate everolimus	0.98 (0.67-1.43)		0.8997
		Type of induction			
12 mo	Best case	ATG (antithymocyte globulin) vs basiliximab (Simulect)	1.47 (0.93-2.33)		0.0976
		Age at transplantation (y)		0.01 (0.01)	0.4389
		BMI			
		<25 vs ≥25 kg/m ²	0.64 (0.44-0.93)		0.0184
		Center size			
		>10 vs ≤10 patients	1.58 (0.80-3.13)		0.1871
		Diabetes mellitus			
		Yes vs no	0.84 (0.43-1.64)		0.6161
12 mo	Best case	Treatment arm			
		Delayed everolimus vs immediate everolimus	0.97 (0.67-1.40)		0.8667
		Type of induction			
12 mo	Best case	ATG (antithymocyte globulin) vs basiliximab (Simulect)	1.40 (0.90-2.18)		0.1403

Cox analysis modeled the risk to the first wound healing complication during the 3- and 12-mo periods. Since the EVR dosage was set to 1.5 mg daily in all study populations (for both arms) and 80% of patients were within the protocol target blood levels range (namely 3–8 ng/mL), the multivariate Cox regression was not fitted, considering either the EVR set dose or EVR blood levels >8 ng/mL as a risk factor (too few cases to justify the analysis); a posthoc analysis of the incidence of wound events between patients out of C₀-EVR target level (ie, above or below 3–8 ng/mL) at any time during the 3 mo of the study vs patients who remained into target level at each time point (in both arms) showed no differences between patients' target level IN vs target level OUT. The incidence of 3 mo BPAR rate/corticosteroid bolus use was 5% (n: 20/383; IE: 11; DE: 9); thus, it was not considered in the Cox model. According to the best-case scenario approach, "failure" (≥1 healing complication) consisted of one of the following cases: wound complication, missing information about wound complications for patients who discontinued the study due to "death" or "graft loss," and study discontinuation due to "death" or "graft loss" for patients who did not attend the month 12 follow-up visit. According to the worst-case scenario, "failure" (≥1 healing complication) consisted of one of the following cases: wound complication, missing information about wound complications, and study discontinuation due to any reason for patients who did not undergo the month 12 follow-up visit. Failure time was defined by the date of first wound complication or that of last visit performed.

ATG, antithymocyte globuline; BMI, body mass index; BPAR, biopsy-proven acute rejection; C₀, immunosuppression blood levels, before morning dose; CI, confidence interval; DE, delayed everolimus; EVR, everolimus; HR, hazard ratio; IE, immediate everolimus; WHC, wound healing complications.

in the DE arm in order, we argue, to avoid an allograft rejection after a decrease in the dose of CNI and the discontinuation of MPA.

The main drawback of the present study is that about 20% of patients in the IE arm and 25% in the DE arm did not complete the CORE-phase study, resulting in a

TABLE 5.
Secondary efficacy endpoint at 3 and 12 mo: ITT population

	IE N = 193		DE N = 190		Log-rank P	Cox proportional hazard model: DE vs IE	
	N (%)	95% CI	N (%)	95% CI		HR (95% CI)	P
3 mo							
Treatment failure (worst-case scenario)	22 (11.4)	7-16	40 (21.0)	15-27	0.0591	1.54 (0.90-2.64)	0.1129
Treatment failure (best-case scenario)	16 (8.3)	4-12	13 (6.8)	3-10	0.5731	0.63 (0.29-1.39)	0.2527
BPAR	11 (5.7)	2-9	9 (4.7)	2-8	0.6539	0.74 (0.30-1.85)	0.5232
D	2 (1.0)	0-2	3 (1.6)	0-3	0.6565	0.55 (0.05-6.30)	0.6326
D/GL	7 (3.6)	1-6	4 (2.1)	0-4	0.3634	0.28 (0.06-1.34)	0.1116
GL	5 (2.6)	0-5	1 (0.5)	0-2	0.1027	0.19 (0.02-1.61)	0.1269
12 mo							
Treatment failure (worst-case scenario)	31 (16.1)	11-21	48 (25.3)	19-31	0.0565	1.43 (0.90-2.27)	0.1291
Treatment failure (best-case scenario)	25 (12.9)	8-18	21 (11.0)	7-16	0.5546	0.69 (0.37-1.27)	0.2302
BPAR	19 (9.8)	6-14	16 (8.4)	4-12	0.6110	0.76 (0.38-1.52)	0.4338
D	2 (1.0)	0-2	3 (1.6)	0-3	0.6565	0.55 (0.05-6.30)	0.6326
D/GL	8 (4.1)	1-7	6 (3.2)	1-6	0.6085	0.48 (0.15-1.61)	0.2372
GL	6 (3.1)	1-6	3 (1.6)	0-3	0.3337	0.49 (0.12-1.95)	0.3084

In the Cox proportional hazard model, the following confounding factors were included: age at KT, BMI (<25 vs ≥25), center dimension (>10 vs ≤10), presence of diabetes mellitus, and induction (ATG vs basiliximab).
ATG, antithymocyte globuline; BMI, body mass index; BPAR, biopsy-proven acute rejection; CI, confidence interval; D, death; DE, delayed everolimus; GL, graft loss; HR, hazard ratio; IE, immediate everolimus; ITT, intent-to-treat; KT, kidney transplantation.

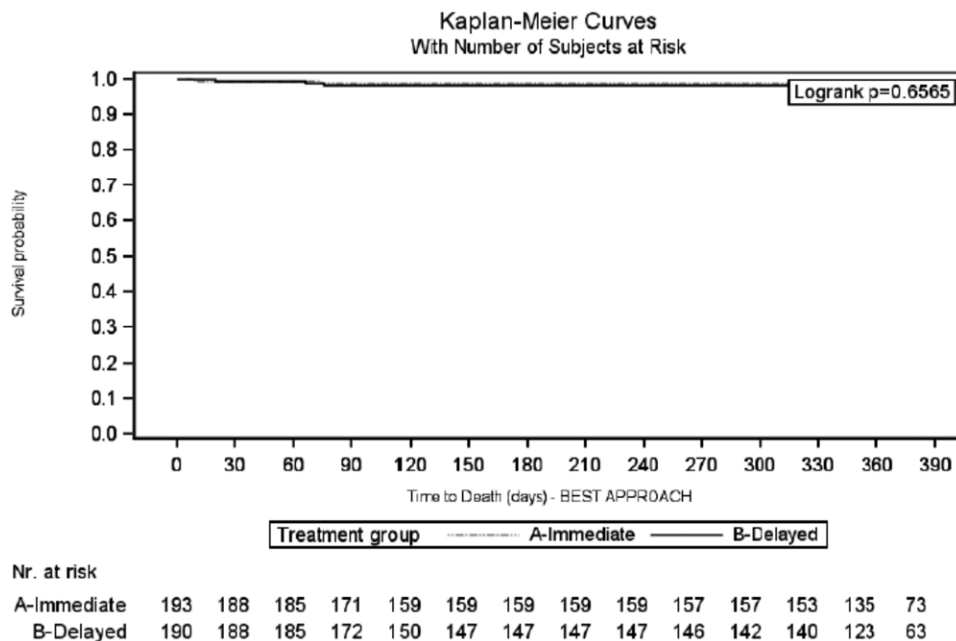


FIGURE 7. Patient survival analysis during the first 12 mo after transplantation: Kaplan-Meier curve (intent-to-treat population).

slightly decrease in the previous sample size powered for the aim of the study. In addition, patients were followed up to 3 months, and some WHCs (ie, lymphocele and incisional hernia) need more time to be evaluated conclusively. This limitations were managed adding a follow-up at 12 months even if, during this observational period, patients were treated—as per clinical practice—in 22 different centers, and therefore, they were monitored less strictly than during the first 3 months. Furthermore, the article is not generalizable to all KT

patients since it lack the presence of severe obese recipients that could sustain WHC.

Although the enrollment of a small proportion of living KT donors could seem to be a limitation of the study, we think that it is representative of the real setting of KT in Italy³⁷ without affecting the study end-points, since only 5% of the study cohort received a kidney from living donors.

In conclusion, treating patients with DE-based IS after KT does not decrease the risk of WHC after KT; hence,

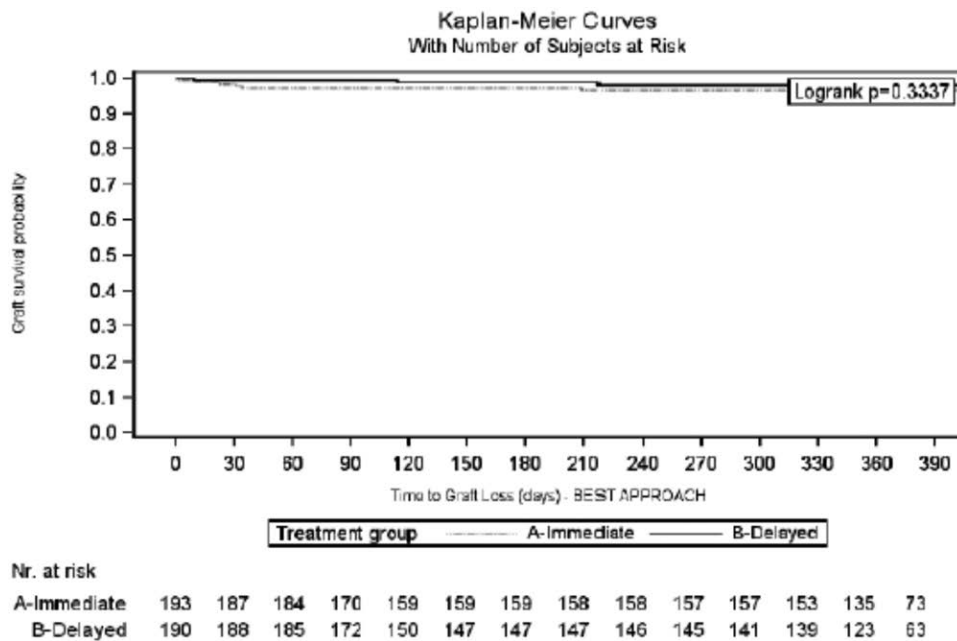


FIGURE 8. Graft survival analysis during the first 12 mo after transplantation: Kaplan-Meier curve (intent-to-treat population).

TABLE 6.

Graft function

	IE N = 193	DE N = 190	P
Follow-up: 3 mo			
DGF ^a (%)	46 (23.8)	60 (31.6)	0.1232 ^b
Days of dialysis, mean (SD)	16.17 (22.8)	9.40 (13.05)	0.2261 ^c
eGFR-MDRD4 (mL/min), mean (SD)	N = 187	N = 183	
At M3	48.82 (23.9)	49.21 (22.7)	—
Changes vs baseline	38.64 (22.4)	39.13 (21.5)	0.9716 ^d
Serum creatinine (mg/dL), mean (SD)	N = 187	N = 183	
At M3	2.02 (1.8)	1.80 (1.0)	—
Changes vs baseline	-4.79 (2.7)	-5.13 (2.3)	0.1191 ^d
Proteinuria, ^e n/N (%)	8/141 (5.7)	8/138 (5.8)	0.07 ^f
Follow-up: 12 mo			
DGF ^a (%)	46 (23.8)	60 (31.6)	0.1232 ^b
Days of dialysis, mean (SD)	16.17 (22.8)	9.40 (13.05)	0.2261 ^c
eGFR-MDRD4 (mL/min), mean (SD)	N = 151	N = 142	
At M12	51.57 (19.3)	51.77 (20.7)	—
Changes vs baseline	41.26 (18.7)	41.56 (19.9)	0.7137 ^d
Serum creatinine (mg/dL), mean (SD)	N = 151	N = 142	
At M12	1.67 (1.0)	1.67 (0.8)	—
Changes vs baseline	-4.96 (2.5)	-5.22 (2.2)	—
Proteinuria, ^e n/N (%)	9/116 (7.8)	11/115 (9.6)	—

^aDGF defined as kidney allograft rejection with final clinical diagnosis "DGF" or the presence of postoperative dialysis started during the first 7 d following the transplant.

^bChi-square test.

^cWilcoxon test.

^dNonparametric ANCOVA model taking into account the following covariates: eGFR at baseline, center size, diabetes mellitus, and age at KT.

^eProteinuria was defined as value >1000 mg/d if assessed in urine collected in 24 h or a value >1.0 if measured on the urine protein/creatinine concentration ratio in a spot urine sample.

^fWald test from logistic regression analysis, taking into account the following factors: age at KT and diabetes mellitus.

DE, delayed everolimus; DGF, delayed graft function; eGFR, estimated glomerular filtration rate; IE, immediate everolimus; KT, kidney transplantation; M, month; MDRD, modification of diet in renal diseases; SD, standard deviation.

de novo EVR does not expose KT recipients to a higher risk of WHC and also maintains a good efficacy and safety profile. Further studies are needed to confirm the clear role

of ATG on WHC, since in the current trial a risk factor was considered only when a worst-case scenario analysis was fitted.

TABLE 7.
Concomitant medications

Overall	IE N = 193	DE N = 190
Antibiotic prophylaxis (%)	192 (99.48)	186 (97.89)
Mean (SD), d	67.68 (36.49)	65.99 (37.84)
Lipid-modifying agents (%)	74 (38.34)	67 (35.26)
ARBs (%)	12 (6.22)	26 (13.68)
α2-Andrenergic (%)	55 (28.50)	65 (34.21)
β-Blockers (%)	99 (51.30)	80 (42.11)
Calcium channel blockers (%)	105 (54.40)	96 (50.53)
Diuretics (%)	153 (79.27)	146 (76.84)

ARBs, angiotensin receptor blockers; DE, delayed everolimus; IE, immediate everolimus.

TABLE 8.
Adverse events

	IE N = 193 N (%)	DE N = 190 N (%)	P
≥1 AEs/infections	171 (88.6)	174 (91.6)	0.42
≥1 SAEs/infections	73 (37.8)	61 (32.1)	0.29
Permanently discontinued due to AEs/infections	29 (15)	21 (11)	0.32
Temporarily discontinued due to AEs/infections	18 (9.3)	23(12.1)	0.47
≥1 infection	73 (37.8)	69 (36.3)	0.84
Most frequent AEs/infections (≥5% in any arms)			
Anemia	70 (36.3)	60 (31.6)	0.39
Leukopenia	9 (4.7)	15 (7.9)	0.27
Abdominal pain	14 (7.2)	16 (8.4)	0.81
Constipation	12 (6.2)	13 (6.8)	0.97
Nausea	4 (2)	14 (7.4)	0.03
Edema peripheral	18 (9.3)	14 (7.4)	0.61
Pyrexia	19 (9.8)	15 (7.9)	0.62
Cytomegalovirus infection	14 (6.7)	19 (10.0)	0.33
Urinary tract infection	38 (19.7)	39 (20.5)	0.94
Hypercholesterolemia	20 (10)	9 (4.7)	0.06
Hypertriglyceridemia	14 (7.2)	9 (4.7)	0.41
Hyperuricemia	21 (10.9)	25 (13)	0.60
Hematuria	14 (7.2)	3 (1.6)	0.01
Hypertension	34 (17.6)	30 (15.8)	0.73
New-onset IDDM	5 (3.1)	6 (4.1)	0.76

P value was calculated by means of chi-square test.

AE, adverse event; DE, delayed everolimus; IDDM, insulin-dependent diabetes mellitus; IE, immediate everolimus; SAE, serious adverse event.

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