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ORIGINAL ARTICLE

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Preformed T cell alloimmunity and HLA eplet mismatch to guide immunosuppression minimization with tacrolimus monotherapy in kidney transplantation: Results of the CELLIMIN trial

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Abbreviations: ABMR, antibody-mediated rejection; BL, borderline lesions; BPAR, biopsy-proven acute rejection; DSA, donor-specific alloantibodies; DSMB, Data Safety Monitoring Board; E−, ELISPOT negative; E+, ELISPOT positive; eGFR, estimated glomerular filtration rate; HLA, Human Leukocyte Antigens; IFN-γ ELISPOT, Interferon gamma Enzyme-linked ImmunoSpot; ITT, intention-to-treat; LI, low immunosuppression (tacrolimus monotherapy); MFI, mean fluorescence intensity; MM, mismatches; MMF, mycophenolate mofetil; PP, per protocol; PVAN, polyoma-virus-associated nephropathy; sc-BPAR, subclinical BPAR; SOC, standard of care; SOP, standard operating procedures; TAC, tacrolimus; TCMR, T cell–mediated rejection.

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Personalizing immunosuppression is a major objective in transplantation. Transplant recipients are heterogeneous regarding their immunological memory and primary alloimmune susceptibility. This biomarker-guided trial investigated whether in low immunological-risk kidney transplants without pretransplant DSA and donor-specific T cells assessed by a standardized IFN-γ ELISPOT, low immunosuppression (LI) with tacrolimus monotherapy would be non-inferior regarding 6-month BPAR than tacrolimus-based standard of care (SOC). Due to low recruitment rates, the trial was terminated when 167 patients were enrolled. ELISPOT negatives (E−) were randomized to LI (n = 48) or SOC (n = 53), E+ received the same SOC. Six- and 12-month BPAR rates were higher among LI than SOC/E− (4/35 [13%] vs. 1/43 [2%], p = .15 and 12/48 [25%] vs. 6/53 [11.3%], p = .073, respectively). E+ patients showed similarly high BPAR rates than LI at 6 and 12 months (12/55 [22%] and 13/66 [20%], respectively). These differences were stronger in *per-protocol* analyses. Post-hoc analysis revealed that poor class-II eplet matching, especially DQ, discriminated E− patients, notably E−/LI, developing BPAR (4/28 [14%] low risk vs. 8/20 [40%] high risk, p = .043). Eplet mismatch also predicted anti-class-I (p = .05) and anti-DQ (p < .001) *de novo* DSA. Adverse events were similar, but E−/LI developed fewer viral infections, particularly polyoma-virus-associated nephropathy (p = .021). Preformed T cell alloreactivity and HLA eplet mismatch assessment may refine current baseline immune-risk stratification and guide immunosuppression decision-making in kidney transplantation.

KEYWORDS
biomarker, clinical decision-making, clinical research/practice, clinical trial, immunobiology, immunosuppression/immune modulation, immunosuppressive regimens - minimization/withdrawal, kidney transplantation/nephrology, rejection: acute

1 | INTRODUCTION

Kidney transplantation is the best treatment for end-stage kidney failure as it improves both quality of life and survival, and it is cost-effective.[1] However, despite optimal short-term outcomes, long-term graft and patient survival remain almost unchanged and unsatisfactory, [2] mainly due to chronic immune-mediated graft injury in addition to the adverse effects related to chronic immunosuppressive therapy.[3,4]

Transplant recipients are not a homogeneous population both in terms of immunological experience and susceptibility for *de novo* alloimmune activation against mismatched donor human leukocyte antigens (HLAs).[5] Hence, the implementation of novel immune tools identifying the distinct anti-donor immune risk is warranted to enable safe individualized immunosuppressive strategies while avoiding unnecessary toxic treatments.[6,7]

Current immunological risk assessment prior to transplantation is exclusively based on the detection of preformed circulating donor-specific alloantibodies (DSA), assuming that humoral allosensitization relates to the allospecific T cell memory immune compartment. However, cellular alloreactivity may occur without humoral activation[8] and plays a major role in initiating and mediating allograft rejection.[9-11] Among different attempts to monitor alloreactive T cell memory *ex vivo*, measuring the frequencies of circulating donor-specific IFN-γ-secreting memory T cells using Enzyme-linked ImmunoSpot (ELISPOT) assays has been shown to be feasible[12,13] and capable of assessing the risk of T cell–mediated rejection (TCMR) both in non-human primates[14] and kidney transplant patients.[15-17] Overall, these studies have shown the potential to specifically rule out the rejection risk among transplant candidates without detectable anti-donor T cell alloimmune responses. The data suggest that the IFN-γ ELISPOT assay is a valuable tool that can be used to guide decision-making regarding the rejection risk and the type and burden of immunosuppressive therapy.[18] To date, most of the studies reported are retrospective and based on small, single-center cohorts and no prospective, randomized trials with treatment interventions guided by the ELISPOT assay have been conducted. Therefore, most biomarkers have no direct impact on guidance of immunosuppression.

Within the European FP7 BIO-DrIM (*BIOmarker-Driven personalized IMmunosuppression*) consortium, the CELLIMIN trial (*Prospective donor-specific Cellular alloresponse assessment for Immunosuppression Minimization in de novo renal transplantation*) was designed to evaluate the usefulness of assessing pretransplant donor-reactive T cell memory, using an IFN-γ ELISPOT assay with a validated standardized operational procedure in each center, to identify kidney transplant

candidates that could safely benefit of receiving lower immunosuppressive burden with tacrolimus (TAC) monotherapy soon after transplantation. The feasibility of implementing a new immune assay in clinical transplantation, and a non-inferior hypothesis regarding the incidence of biopsy-proven acute rejection (BPAR) as compared to recipients with the same immune-risk profile receiving current standard of care (SOC) therapy based on TAC, mycophenolate mofetil and prednisone, was tested. The main hypothesis of the trial was that by excluding preformed anti-donor immune memory, both cellular and humoral, TAC monotherapy would be effective enough to abrogate primary anti-donor immune activation while reducing drug-related toxicity within the first year after transplantation.

2 | METHODS

2.1 | Study design

The CELLIMIN trial was a prospective, multi-center, biomarker-driven, randomized trial performed within the European BIO-DRIM research consortium, sponsored by the European Union Seventh Framework Program (FP7-HEALTH-2012-INNOVATION-1, grant agreement nº 305147). Eight kidney transplant centers across Europe participated in the trial, Bellvitge University Hospital (Barcelona, Spain), Charité (Berlin, Germany), Amsterdam University Medical Centers (Amsterdam, the Netherlands), Universitätsklinikum Hamburg-Eppendorf (Hamburg, Germany), Institute for Clinical and Experimental Medicine (Prague, Czech Republic), Centre Hospitalier Universitaire Nantes (Nantes, France), University Hospital Regensburg (Regensburg, Germany), and University Hospital Marqués de Valdecilla (Santander, Spain). Each center participated under the approval of the Europe-wide voluntary harmonization process (VHP). An external Data Safety Monitoring Board (DSMB) was responsible for periodic safety review and guided by predetermined protocol–defined stopping criteria.

The study protocol is available online at https://clinicaltrials.gov/ct2/show/NCT02540395.

2.2 | Participants

Low immunological risk subjects were eligible to participate if >18 years of age and receiving a primary single kidney transplant (inclusion and exclusion criteria are described in Data S1). Enrolment was targeted to 673 patients, with 302 E− transplant patients randomized to low or SOC immunosuppression. However, due to slow patient enrolment, the trial was terminated when 167 were recruited. In all, 101 patients were randomized and followed for 12 months.

All subjects freely gave written informed consent prior to participation, including informed consent for the screening procedures to establish subject eligibility.

2.3 | Procedures

2.3.1 | Study treatments

Transplant patients were first allocated into two groups according to their pretransplant donor-specific IFN-γ ELISPOT result (flow chart of the study in Figure 1).

Group I. ELISPOT negative (E−) candidates were randomized to receive:

- **Standard of care immunosuppression (SOC):** Based on current standard of care therapy consisting in TAC to achieve a 4–8 ng/ml plasma trough levels, mycophenolate mofetil (MMF) initially 1gr bid and subsequently adjusted according to the subjects tolerance, and 500 mg methylprednisolone perioperatively to continue with oral prednisone (20 mg/day the first 2 weeks and tapered not less than 5 mg/day at 4 weeks posttransplant).
- **Low immunosuppression (LI):** Based on TAC monotherapy to achieve TAC 8–10 ng/ml plasma trough levels during the first 4 weeks and 6–8 ng/ml thereafter, MMF (1 g bid) during the first week posttransplant and stopped thereafter, and 500 mg methylprednisolone perioperatively to continue with oral prednisone 20 mg/day the first 2 weeks and tapered to 5 mg/day from month 1 to month 2 when finally discontinued.

Group II. ELISPOT positive (E+) transplant candidates received the same current standard of care immunosuppressive regimen than group E−/SOC.

All patients received two doses of basiliximab (20 mg) at days 0 and 4 after transplantation.

Patients were followed up for a total of 12 months for secondary outcome measures.

Types of BPAR rescue therapies were provided according to the respective standard of care in each center: for TCMR: Banff <IIA TCMR, 3 doses of 500 mg of 6-Methyl prednisolone; Banff >IB TCMR, 3–5 doses of 1 mg/kg Thymoglobulin. For ABMR: plasmapheresis/immunoadsorption with IVIG or Rituximab. MMF and prednisone were reintroduced in all patients developing rejection under TAC monotherapy.

2.3.2 | Histology assessment

For cause biopsies were performed in case of either lack of graft function improvement or sudden graft dysfunction by means of serum creatinine, estimated glomerular filtration rate (eGFR) or proteinuria and rejection was defined as *clinical BPAR*. *Surveillance* biopsies were planned at 3 and 12 months after transplantation and were defined as graft biopsies performed in patients with serum creatinine <300 μmol/L; proteinuria <1 g/24 h and stable renal function (variability of serum creatinine of <15% during 2 weeks before and after biopsy) and rejection was defined as *subclinical BPAR*. All core biopsy samples were analyzed by expert transplant pathologists from each

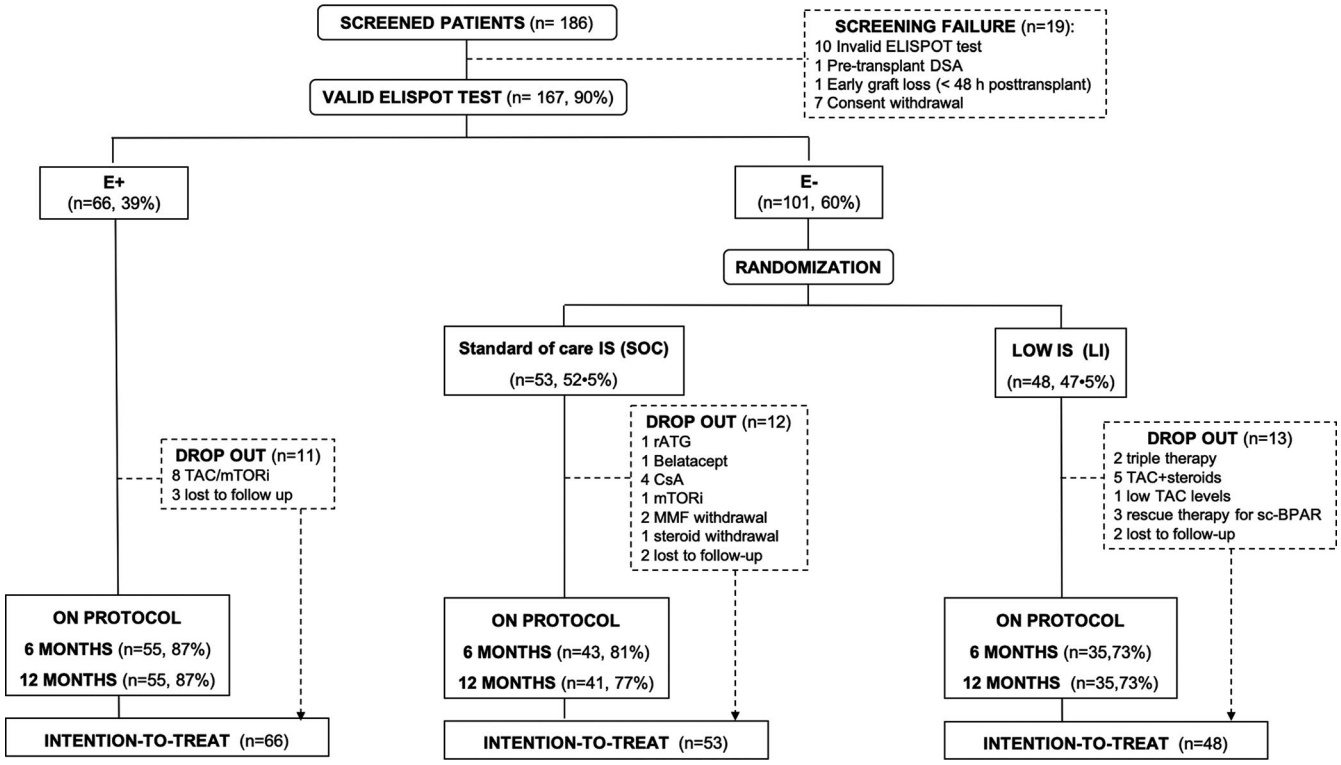

FIGURE 1 Flow chart of the study

participating center and graded following the Banff 2017 classification.[19] In total, 113 (69%) patients underwent a surveillance biopsy at 3 and/or 12 months and 106 (63.5%) were evaluable for its diagnosis; 35 (66%), 38 (79%), and 33 (50%) in the E−/SOC, E−/LI, and E+ groups, respectively.

2.3.3 | Laboratory studies

Donor-specific IFN-γ Enzyme-Linked ImmunoSpot (ELISPOT) assays

Supplemental methods report recipient and donor peripheral blood mononuclear cell and splenocytes standard operating procedures (SOP) used as well as a detailed description of the donor-specific IFN-γ ELISPOT assays, which was extensively cross-validated between centers.[12] A result of ≥25 IFN-γ ELISpots/3x10^5 PBMC was considered as a POSITIVE test, whereas <25 as NEGATIVE.

HLA typing and molecular mismatches

Donor and recipient HLA class-I (A, B, and C) and class-II (DRB1, DQB1, and DQA1) high-resolution typing was performed with NGS technology in 154/167 (92%) donor/recipient pairs on a MiSeq platform (Illumina, San Diego, California). In the remaining patients, DNA-based low-resolution HLA typing was performed with sequence-specific primers (SSP) and were extrapolated to high-resolution using a previously validated computational method based on haplotype frequency Tables.[20,21] Donor/recipient HLA eplet mismatches (both non-verified and antibody-verified) were determined by the last versions of the HLAMatchmaker software (HLA-ABC Eplet Matching V3.1 and DRDQDP Eplet Matching Program V3.1). Results were also compared with the previous HLA-Matchmaker software version (HLA-ABC Eplet Matching Version 2 and DRDQDP Eplet Matching Program V2.2).[22]

Anti-HLA antibody determination

A Single-Antigen Class-I and Class-II flow beads-assay kit was used (Lifecodes, Immucor, Stanford, CA) to monitor serum anti-HLA antibodies at baseline and at 12 months after transplantation. All beads showing a normalized mean fluorescence intensity (MFI) >500 were considered positive if (MFI/MFI lowest bead)>5.

2.4 | Outcomes

The primary study endpoint was to demonstrate in a per-protocol analysis, non-inferiority rates of BPAR, excluding borderline lesions, in *for cause* biopsies at 6 months after transplantation, allowing a non-inferiority margin of 10% (full description in Data S1).

Secondary outcomes analyzed as a post-hoc analysis were as follows: incidence of clinical and subclinical BPAR both per protocol and intention-to-treat, also taking into account the E+ group of patients, differences in eGFR, dnDSA, graft and patient survival and impact of donor/recipient HLA molecular mismatches on BPAR and dnDSA between groups at 12 months of follow-up.

Incidence of adverse events, serious adverse events, infections, and malignancies was recorded in each center.

TABLE 1 Main clinical and demographic characteristics of the patients of the study

	E−/SOC (*n* = 53)	E−/LI (*n* = 48)	E+ (*n* = 66)	*p* value
Recipient age (years)	53.51 ± 12.81	54.68 ± 14.11	53.88 ± 13.97	.907
Recipient sex				
Female	12 (22.6)	16 (33.3)	19 (29.2)	.481
Male	41 (77.4)	32 (66.7)	46 (70.8)	
Recipient ethnicity				
Caucasian	50 (94.3)	45 (93.75)	46 (97.9)	.574
No Caucasian	3 (5.7)	3 (6.25)	1 (2.1)	
Cause of end-stage renal disease				
Glomerulonephritis	10 (18.9)	15 (31.9)	17 (26.2)	.514
Vascular	3 (5.7)	3 (6.4)	8 (12.3)	
Diabetes Mellitus	12 (22.6)	4 (8.5)	7 (10.8)	
Polycystic kidney disease	12 (22.6)	10 (21.3)	13 (20)	
Unknown	9 (17)	10 (21.3)	15 (23.1)	
Others	7 (13.2)	5 (10.4)	5 (7.5)	
Type of donor				
Living	28 (52.8)	26 (54.2)	35 (53)	.990
Living-related, yes	11 (20.8)	11 (22.9)	25 (37.9)	.384
Preemptive transplantation	13 (24.5)	36 (75)	40 (60.6)	.133
Time on dialysis (months)	41.20 ± 50.44	34.50 ± 51.06	23.06 ± 28.30	.088
CMV prophylaxis, yes	15 (28.8)	14 (31.1)	23 (39)	.492
Baseline Panel Reactive Antibodies	0.45 ± 2.43	0.0 ± 0	0.23 ± 1.14	.469
Preformed DSA	0 (0)	0 (0)	0 (0)	1.000
HLA allelic MM	5.58 ± 2.59	6.77 ± 1.77	7.24 ± 2.3	.001*
Class I	3.57 ± 1.69	4.33 ± 1.19	4.24 ± 1.59	.03
Class II	2.02 ± 1.29	2.44 ± 1.09	3.00 ± 1.07	<.001
Pretransplant donor-specific IFN-γ ELISpots (per 3 x 10^5 PBMC)	7.75 ± 6.82	7.67 ± 7.03	80.02 ± 84.13	<.001**
Delayed graft function	14 (26)	7 (15)	7 (11)	.137
Kidney graft loss	3 (5.8)	0 (0)	1 (1.6)	.155
Patient death	2 (3.8)	1 (2.1)	1 (1.5)	.704

Abbreviations: CMV, cytomegalovirus; E−/LI, donor-specific ELISPOT negative/low immunosuppression; E−/SOC, donor-specific ELISPOT negative/standard of care immunosuppression; E+, donor-specific ELISPOT positive; HLA, human leukocyte antigen; MM, mismatches.

Data are mean ± SD or n (%).

*Total HLA allelic MM: E−/SOC vs. E−/LI $p = .036$; E−/SOC vs. E+ $p = .001$; E−/LI vs. E+ $p = .55$. Class I HLA allelic MM: E−/SOC vs. E−/LI $p = .043$; E−/SOC vs. E+ $p = .058$; E−/LI vs. E+ $p = .95$. Class II HLA allelic MM: E−/SOC vs. E−/LI $p = .19$; E−/SOC vs. E+ $p < .001$; E−/LI vs. E+ $p = .038$. ; **Pretransplant donor-specific IFN-γ ELISpots: E−/SOC vs. E−/LI $p = 1.000$; E−/SOC vs. E+ $p < .001$; E−/LI vs. E+ $p < .001$.

2.5 | Statistical analysis

The study design and sample size calculation are depicted in detail in Data S1. Since the primary study endpoint could not be achieved, a number of clinically relevant outcomes were analyzed as a post-hoc analysis. Comparisons of the primary and secondary outcomes across ELISPOT subgroups were done using a chi-square test for qualitative data and T-test or Wilcoxon signed-ranked test for the comparison of continuous secondary outcomes. The time-dependent association of the variables assessed with BPAR was

studied using Kaplan–Meier plots and log-rank test. Receiver operating characteristic (ROC) curve analysis was used to evaluate most sensitive and specific donor/recipient HLA molecular mismatch cutoffs predicting BPAR. The statistical significance level was defined as two-tailed $p < .05$. Statistical analyses were performed with IBM SPSS Statistics, version 26 and GraphPad Prism version 6.0 (GraphPad Software).

3 | RESULTS

3.1 | Patients of the study and main clinical outcomes

As described in Figure 1, a total of 186 patients were screened and 167 enrolled between December 8, 2015 and October 23, 2018; 66 (39%) were Elispot positive (E+), whereas 101 (60%) E− and were subsequently randomized to receive either lower immunosuppression (LI) with TAC monotherapy (n = 48, 47.5%) or current SOC (n = 53, 52.5%). Despite the high recruitment priority established in each center, the stringent low immunological risk inclusion criteria led to insufficient recruitment rates. Thus, in agreement with the DSMB, the trial was terminated.

Main baseline clinical characteristics were not different between groups (Table 1), but E+ showed higher HLA allelic mismatches and, as per study design, higher donor-reactive IFN-γ ELISpots. There were four (2.3%) graft losses, three within the E−/SOC group (two because of obstructive nephropathy and one for polyoma-virus-associated nephropathy), and one in the E+ because of chronic antibody-mediated rejection (cABMR), and there were four (2.3%) deaths (two E−/SOC patients because of a bacterial sepsis and lung cancer, one in the E−/LI group due to multiple myeloma and one in the E+ group because of sudden cardiac arrest). At 6 months, 133 (80%) patients remained on protocol and 131 (78%) at 12 months; 41 (77%) in the E−/SOC, 35 (73%) E−/LI, and 55 (87%) E+. Main causes of dropout are described in Table S1.

As per study protocol, plasma TAC trough levels were significantly higher among E−/LI than E−/SOC and E+ patients until month 2, whereas at 3, 6, and 12 months, all groups showed similar exposure (Table S2).

3.1.1 | Incidence of BPAR in the trial

At 6 months, 21 (12.5%) patients developed clinical BPAR, 28 (17%) when including Banff borderline lesions. At 12 months, three additional clinical BPAR occurred (Banff≥IA); thus, a total of 31 (18.5%) patients developed BPAR during the 12-month follow-up (Table S3). While all BPAR within the E− groups were TCMR, there were six ABMR among E+ patients. Of the total BPAR, six occurred in patients not on protocol (three E−/LI arm [1 BL and 2 Banff≥IA] and three among E−/SOC group, all Banff≥IA). In total, 106 patients underwent a 3/12 months protocol biopsy with evaluable material. In all, 17 (16%) patients developed Banff≥IA subclinical BPAR (sc-BPAR) and 10 (9.4%) showed BL changes (Table S4). 6/17 (35.3%) patients with sc-BPAR and two out of 10 (20%) showing sc-BL changes had previously developed clinical BPAR. TAC trough levels and intrapatient variability (IPV) prior to clinical or subclinical BPAR was not associated with higher rejection rates, both globally and within each study group.

3.1.2 | Primary study endpoint

The analysis of the primary study endpoint evaluating the incidence of BPAR at 6 months between E−/SOC and E−/LI groups, excluding BL lesions, showed no statistically significant differences between groups (1/43 [2%] vs. 4/35 [13%], p = .16, respectively) (Table 2). Six-month cumulative incidences of BPAR were not different between the two E− groups both in PP and ITT analyses (Figure 2A-B).

3.2 | Post-hoc analysis of main clinical outcomes between all study groups

3.2.1 | Incidence of clinical and subclinical BPAR

When E+ patients were also analyzed, at 6 months, E+ showed significantly higher BPAR (both with and without BL lesions) than E−/SOC patients (Table 2). Similarly, at 12 months, BPAR rates were significantly higher within E+ and E−/LI patients as compared to E−/SOC, especially in patients remaining on protocol. 12-month cumulative BPAR between the three groups showed the same differences both when assessed PP or ITT (Figure 2C-D).

Likewise clinical BPAR, both E+ and E−/LI groups developed significantly higher incidence of sc-BPAR than E−/SOC (Table 2).

3.2.2 | Twelve-month de novo DSA (dnDSA)

At 12 months, 149 (89%) patients were tested for anti-HLA antibodies; 47 (88%) among E−/SOC, 43 (89%) within E−/LI and 59(89%) among E+ patients (Table S4). In all, 17 dnDSA were detected among 11 (7.4%) patients, 6 class I (3 anti-A and 3 anti-B), and 11 class II (7 anti-DQ and 4 anti-DR). As shown in Table 2, while no differences were observed regarding total dnDSA between the three groups, E+ patients displayed higher class-II dnDSA than the other groups.

3.2.3 | Kidney graft function progression

After month 2, E−/LI patients displayed lower eGFR than E−/SOC and E+ recipients until month 12 after transplantation (Figure 3), although these differences were not significant when only patients

TABLE 2 Main study outcomes between the different study groups

	E−/SOC	E−/LI	E+	E−/LI vs. E−/SOC	E+ vs. E−/LI	E+ vs. E−/SOC
6-mo PP (*n* = 133)	*n* = 43	*n* = 35	n = 55	*p* values		
BPAR (excluding BL)[a]	1 (2)	4 (13)	12 (22)	.158[b]	0.394	0.006
BPAR	3 (7)	8 (23)	12 (22)	.056	0.908	0.051
12-mo PP (*n* = 131)	*n* = 41	*n* = 35	*n* = 55			
BPAR	3 (7)	9 (26)	13 (24)	.055	0.823	0.051
BPAR ITT (*n* = 167)	*n* = 53	*n* = 48	*n* = 66	*p* values		
6-mo BPAR	5 (9.5)	11 (23)	12 (18)	.064	0.534	0.175
12-mo BPAR	6 (11.3)	12 (25)	13 (20)	.073	0.499	0.213
Sc-BPAR	1 (2.9)	10 (26.3)	6 (18.2)	.005	0.413	0.038
Sc-BL	4 (11.4)	4 (10.5)	2 (6.1)	.902	0.500	0.435
De novo DSA	*n* = 47	*n* = 43	*n* = 59	*p* values		
Total dnDSA	1 (2)	3 (7)	7 (12)	.345	0.513	0.074
Class-I dnDSA	1 (2)	3 (7)	2 (3.4)	.345	0.648	1.000
Class-II dnDSA	0	1 (2)	7 (12)	.478	0.134	0.017

Abbreviations: BL, Banff borderline lesions; BPAR, biopsy-proven acute rejection; dnDSA, *de novo* donor-specific antibodies; E−/LI, donor-specific ELISPOT negative/low immunosuppression; E−/SOC, donor-specific ELISPOT negative/standard of care immunosuppression; E+, donor-specific ELISPOT positive; ITT, intention-to-treat; mo, months; PP, per protocol; Sc-BPAR, subclinical biopsy-proven acute rejection.

All BPAR analyses include Banff borderline (BL) lesions but the primary study endpoint.

Data are mean ± SD or n (%).

[a]Patients having received rescue therapy due to borderline BPAR prior to 6 months (*n* = 4, in the E−/LI and *n* = 2 in the E−/SOC) were excluded of this per protocol analysis.

[b] Statistical comparison of the primary endpoint of the CELLIMIN trial.

on protocol were analyzed. 12-month eGFR was lower among E−/LI patients developing BPAR as compared to those that did not. These differences were not observed in the other two groups. Subclinical BPAR did not impact on 12-month eGFR in any study group (Figure S1).

3.3 | HLA eplet mismatching and *de novo* alloimmune activation

We next assessed the impact of donor/recipient HLAMatchmaker eplet mismatches on main immune-mediated events between the distinct study groups. Similar to HLA allele mismatches, E− patients showed lower eplet mismatches as compared to E+ (Table S5).

3.3.1 | HLA eplet mismatching and incidence of BPAR

Mean class-II eplet mismatches (MM) (DRB1+DQ), and particularly at DQ locus, were significantly higher in patients developing BPAR than in those that did not (Figure 4

). However, these differences were only observed among the two E− study groups. A threshold of DQ (A1/B1) eplet mismatches ≥10 defined high eplet risk for BPAR with the highest accuracy within all E− patients (AUC = 0.733; 95% CI 0.612–0.853) (Figure S2). As illustrated in Figure 5A, high-risk DQ eplet mismatching was associated with higher BPAR rates only among E− patients, and particularly among E−/LI (6/28 [21%] in E+, 1/28 [4%] in E−/SOC and 4/28 [13%] in E−/LI, $p = .137$ in low-risk eplet patients, whereas 7/38 [18%] in E+, 5/25 [20%] in E−/SOC and 8/20 [40%] in E−/LI, $p = .16$ within the high-risk eplet group). When we analyzed the association between eplet MM risk score and global BPAR rates (clinical and/or subclinical), similarly higher rates of BPAR and/or sc-BPAR were observed among E− patients, especially within E−/LI patients, with high-risk eplet score ($p = .07$) (Figure 5b).

DQ eplet MM risk score at the single donor molecule identified three risk groups (low risk: 0 DQ MM, intermediate: 1–5; high: ≥6 DQ MM), although with lower predictive accuracy (AUC = 0.684; 95% CI 0.59–0.78, $p < .001$). High-risk patients did also display significantly higher BPAR rates than low and intermediate-risk groups within E−/SOC and E−/LI patients (1/25 [4%] vs. 5/28 [18%], $p = .19$ in E−/SOC and 1/14 [7%] vs. 11/34 [32%], $p = .06$ in E−/LI) but not in E+ (4/21 [19%] vs. 9/45 [20%], $p = 1$].

3.3.2 | HLA eplet mismatching and de novo DSA

Patients with anti-class-I and anti-DQ dnDSA displayed significantly higher class I and DQB1 single molecule eplet mismatches

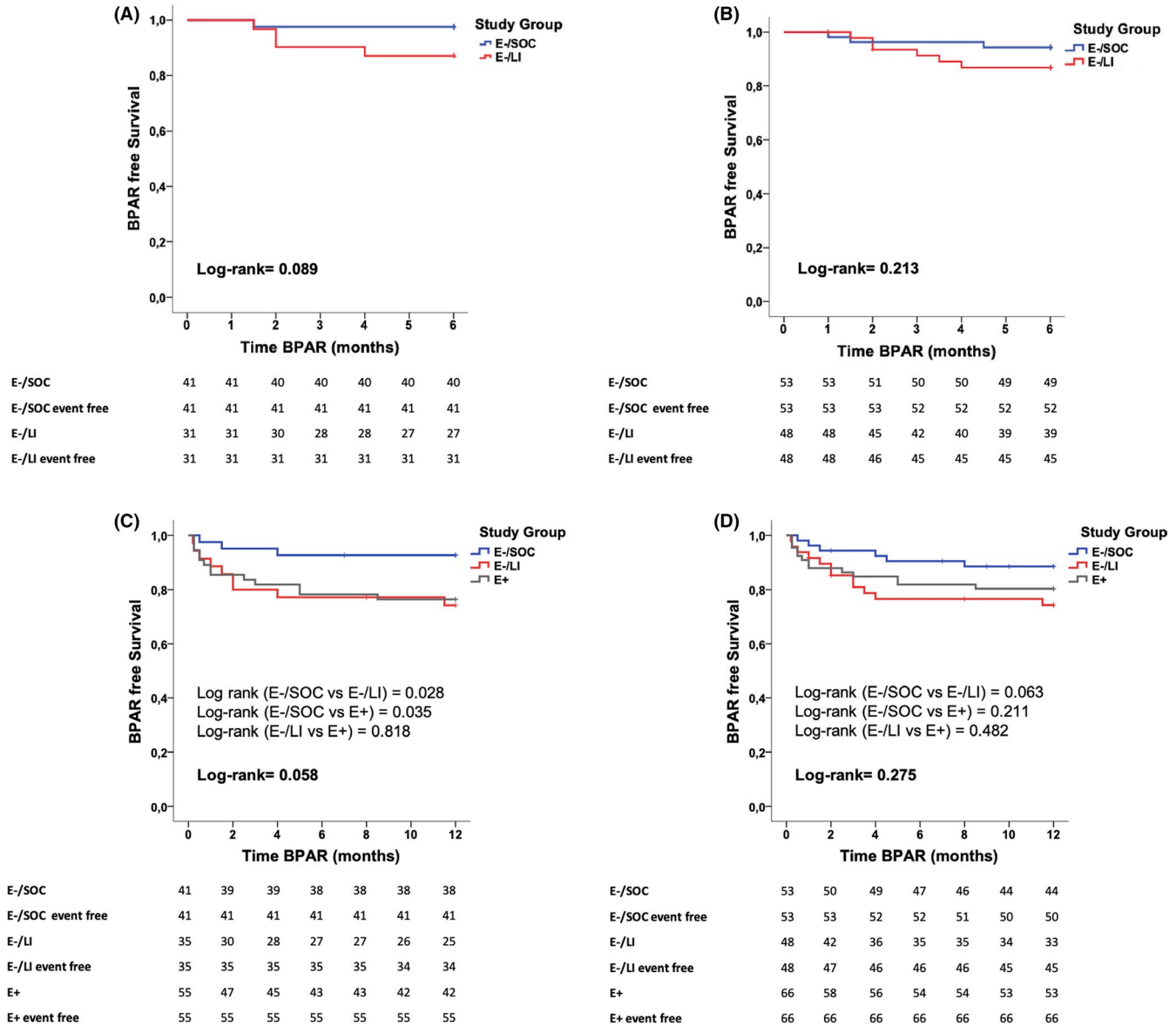

FIGURE 2 BPAR rates between the study groups in all patients and in patients on protocol at 6 and 12 months. (A) Six-month Kaplan–Meier BPAR-free (excluding BL lesions) survival curves in patients on protocol (primary endpoint) (n = 72) in the two E− groups (log rank = 0.089). (B) Six-month Kaplan–Meier BPAR-free (excluding BL lesions) survival curves in all patients (intention to treat) (n = 101) in the two E− groups (log rank = 0.213). (C) Twelve-month Kaplan–Meier BPAR-free (including BL lesions) survival curves in patients on protocol (n = 131) according to the three different study groups (log rank = 0.058). Log rank (E−/SOC vs. E−/LI) =0.028; log rank (E−/SOC vs. E+) =0.035; log rank (E−/LI vs. E+) =0.818. (D) Twelve-month Kaplan–Meier BPAR-free (including BL lesions) survival curves in all patients (intention-to-treat) (n = 167) according to the three different study groups (log rank = 0.275). log rank (E−/SOC vs. E−/LI) =0.063; log rank (E−/SOC vs. E+) =0.211; log rank (E−/LI vs. E+) =0.482

than patients that did not, respectively (14.74 ± 7.04 vs. 19.00 ± 2.89, p = .050 for class I and 5.19 ± 5.16 vs. 13.33 ± 5.09, p < .001for DQ) (Figure S3). Eplet mismatches at the DR locus were not assessed because only four patients developed anti-DR dnDSA.

A high correlation between the number of eplet MM detected with the two most recent HLAMatchmaker algorithms versions (V2 and V3.1) was observed (Spearman Rho >0.9 and p < .001 at all loci). The same impact on main clinical outcomes both BPAR and dnDSA was similarly observed with the two algorithms (data not shown).

3.4 | Safety

The number of adverse and serious adverse events did not differ between the three study groups (Table 3). While the incidence of any kind of infection equally occurred across the three groups, a

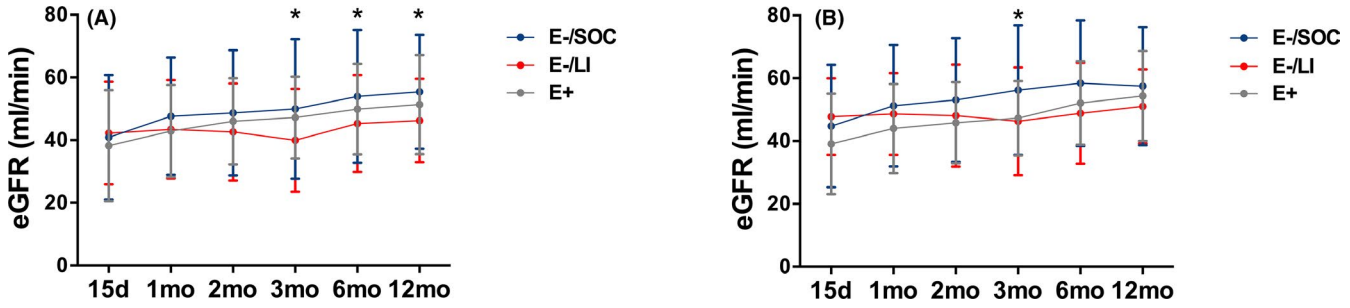

FIGURE 3 Twelve-month eGFR progression between study groups. (A) Twelve-month eGFR progression between study groups in all patients (intention to treat), n = 167. eGFR were 40.88 ± 19.88 vs. 42.26 ± 16.36 vs. 38.21 ± 17.74 ml/min, p = .549 at 15 days; 47.66 ± 18.71 vs. 43.46 ± 15.69 vs. 42.93 ± 14.70 ml/min, p = .266 at 1 month; 48.72 ± 19.98 vs. 42.62 ± 15.51 vs. 46.00 ± 13.79 ml/min, p = .202 at 2 months, 49.95 ± 22.27 vs. 39.97 ± 16.41 vs. 47.20 ± 13.03 ml/min, p = .019 at 3 months, 53.95 ± 21.16 vs. 45.31 ± 15.44 vs. 49.91 ± 14.41 ml/min, p = .078 at 6 months, and 55.44 ± 18.21 vs. 46.25 ± 13.29 vs. 51.36 ± 15.81 ml/min, p = .030 at 12 months in E−/SOC vs. E−/LI vs. E+, respectively. (B) Twelve-month eGFR progression between study groups in patients that were on protocol at 12 months (n = 106). eGFR were 44.77 ± 19.49 vs. 47.80 ± 12.15 vs. 39.11 ± 15.99 ml/min, p = .155 at 15 days; 51.25 ± 19.34 vs. 48.65 ± 12.99 vs. 44.01 ± 14.17 ml/min, p = .135 at 1 month; 53.08 ± 19.71 vs. 48.01 ± 16.23 vs. 45.84 ± 12.95 ml/min, p = .157 at 2 months, 56.25 ± 20.60 vs. 46.32 ± 17.13 vs. 47.28 ± 11.82 ml/min, p = .029 at 3 months, 58.43 ± 20.00 vs. 48.84 ± 16.07 vs. 52.10 ± 13.26 ml/min, p = .069 at 6 months, and 57.48 ± 17.86 vs. 51.04 ± 11.76 vs. 54.36 ± 14.32 ml/min, p = .296 at 12 months in E−/SOC vs. E−/LI vs. E+, respectively

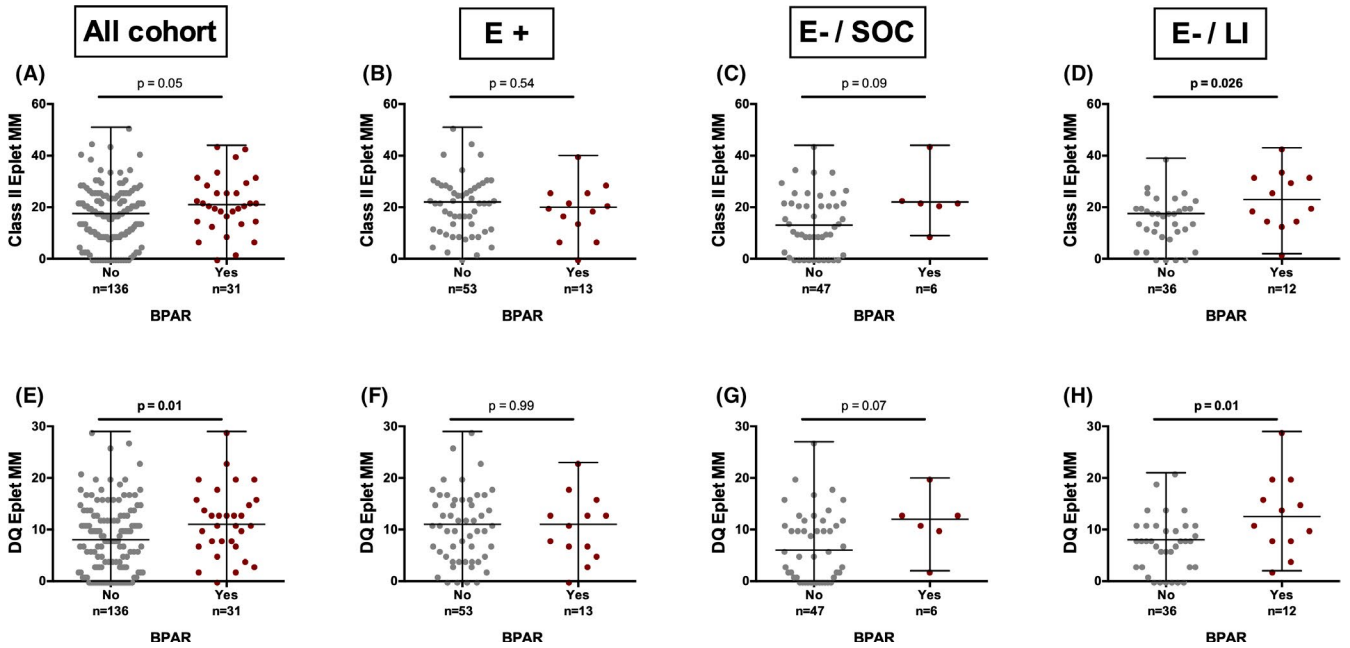

FIGURE 4 Mean donor/recipient HLA class II and DQ eplet MM between patients with or without BPAR. (A) Mean donor/recipient HLA class-II eplet MM and BPAR in all patients: 21.61 ± 10.88 in BPAR patients vs. 17.12 ± 11.16 in patients not experiencing BPAR, p = .05. (B) Mean donor/recipient HLA class-II eplet MM and BPAR in E+ patients: 19.08 ± 10.53 in BPAR patients vs. 20.91 ± 11.10 in patients not experiencing BPAR, p = .529. (C) Mean donor/recipient HLA class-II eplet MM and BPAR in E−/SOC patients: 23.50 ± 11.32 in BPAR patients vs. 14.25 ± 11.57 in patients not experiencing BPAR, p = .089. (D) Mean donor/recipient HLA class-II eplet MM and BPAR in E−/LI patients: 23 ± 42 in BPAR patients vs. 15.31 ± 9.22 in patients not experiencing BPAR, p = .026. (E) Mean donor/recipient HLA DQ eplet MM and BPAR in all patients: 11.71 ± 6.66 in BPAR patients vs. 8.54 ± 6.63 in patients not experiencing BPAR, p = .015. (F) Mean donor/recipient HLA DQ eplet MM and BPAR in E+ patients: 10.53 ± 6.38 in BPAR patients vs. 10.69 ± 6.87 in patients not experiencing BPAR, p = .987. (G) Mean donor/recipient HLADQ MM and BPAR in E−/SOC patients: 11.50 ± 5.82 in BPAR patients vs. 6.96 ± 6.72 in patients not experiencing BPAR, p = .07. (H) Mean donor/recipient HLA DQ MM and BPAR in E−/LI patients: 13.08 ± 7.59 in BPAR patients vs. 7.42 ± 5.31in patients not experiencing BPAR, p = .015

significantly lower incidence of viral infections, particularly BK viremia and polyoma-virus-associated nephropathy (PVAN) was observed among E−/LI patients (16 [30.2%], 6 [12.5%], and 11 [16.9%], p = .06 for BK viremia and 5 [9.4%], 0 (0%), and 1 [1.5%], p = .02 for PVAN, in E−/SOC, E−/LI, and E+ patients, respectively). No other differences were observed regarding main hematological, cardiovascular or metabolic disorders, or in the incidence of malignancies between study groups.

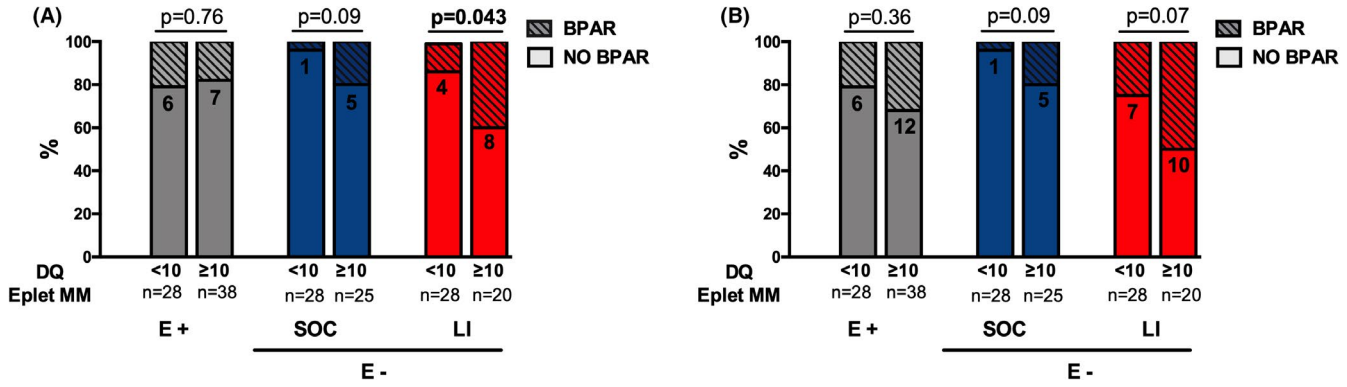

FIGURE 5 Donor/recipient HLA DQ eplet MM risk score for clinical and subclinical BPAR between study groups. (A) Donor/recipient HLA DQ eplet MM risk score for clinical BPAR between study groups. E+ patients: 6/28 (21%) low eplet risk vs. 7/38 (18%) high eplet risk, $p = .76$. E−/SOC patients: 1/28 (4%) low eplet risk vs. 5/25 (20%)high eplet risk, $p = .09$. E−/LI patients: 4/28 (14%) low eplet risk vs. 8/20 (40%) high eplet risk, $p = .043$. (B) Donor/recipient HLA DQ eplet MM risk score for clinical+subclinical BPAR between study groups. E+ patients: 6/28 (21%) low eplet risk vs. 12/38 (31%) high eplet risk, $p = .36$. E−/SOC patients: 1/28 (4%) low eplet risk vs. 5/25 (20%) high eplet risk, $p = .09$. E−/LI patients: 7/28 (25%) low eplet risk vs. 10/20 (50%) high eplet risk, $p = .07$

4 | DISCUSSION

The CELLIMIN trial was designed to evaluate the hypothesis of whether immune-monitoring preformed anti-donor T cell immune memory, posttransplant immunosuppression minimization with TAC monotherapy would be effective enough while reducing drug-related toxicities. Although we were unable to reach the statistical power required to evaluate our primary hypothesis, our findings reveal interesting novel information. First, we show that implementing a novel cellular-based immune assay measuring donor-reactive memory/effector IFN-γ-producing T cells is safe and feasible in real clinical practice. However, the higher BPAR rates observed among the low immunologic risk group receiving TAC monotherapy (E−/LI) as compared to low-risk patients receiving current standard of care therapy (E−/SOC), especially when also taking into account Banff BL lesions (25% vs. 11%), outweighs any potential benefit of maintaining E− kidney transplant recipients on TAC monotherapy on the solely basis of monitoring pretransplant anti-donor T cell memory and serum DSA. Nonetheless, we found that among patients receiving the same SOC therapy, E− transplants outperformed significantly the E+ group regarding BPAR rates, *dn*DSA formation and eGFR, suggesting the value of the ELISPOT immune-risk stratification. Moreover, and as hypothesized, patients on TAC monotherapy did benefit of lower viral infection rates as compared to patients on a triple drug-based regimen.

Since the exceeding BPAR rates among E−/LI patients as compared to E−/SOC could not be explained by preformed anti-donor T cell memory, we hypothesized whether they could rather be due to poor donor/recipient HLA eplet matching in the context of low immunosuppression. Unlike E+ transplants, E− patients with high-risk DQ eplet mismatch score more frequently developed BPAR, an effect that was even more evident within E− patients on TAC monotherapy. Indeed, while only 4/28 (14%) and 7/28(25%) of E−/LI patients with low-risk eplet score developed clinical and subclinical BPAR, respectively, up to 8/20 (40%) and 10/20 (50%) of those with a high-risk eplet score did. These findings are in agreement with previous and recent studies showing the capacity of HLA molecular mismatching predicting primary alloimmune activation, and especially in patients receiving low or insufficient immunosuppression.[23-26] Moreover, and as previously reported,[27,28] we found a close association between a poor donor/recipient HLA eplet matching at each respective locus and *dn*DSA formation. Altogether, these data suggest that adding the analysis of HLA eplet mismatching to preformed anti-donor T- and B-cell memory seems to have the potential to identify a relevant proportion of transplant recipients (25%) that could successfully receive lower immunosuppression with TAC monotherapy until 1 year after transplantation.

The assessment of preformed anti-donor T cell memory discriminated transplant patients receiving the same SOC immunosuppression who were at higher risk of BPAR. These findings corroborate previous retrospective studies[18,29] and highlight the importance of monitoring preformed T cell memory as these patients could not have been identified using current clinical and epidemiologic factors indicative of low immunological risk, such as first transplant recipients with low cPRA and no DSA. Interestingly, ABMR did only occur within E+ patients and relatively soon after transplantation, a finding suggesting the concomitant presence of anti-donor alloreactive memory B cells despite the absence of detectable DSA in serum.[30] Nevertheless, while the high BPAR rates within E+ patients seem to be predominantly driven by preformed anti-donor T cell memory, the poorer HLA matching of this group of patients, raises concerns on whether these patients might also be at high risk of subsequent primary alloimmune activation in the long term. While we cannot exclude that higher donor/recipient HLA mismatching among E+ patients may be coincidental, our data also suggest that since the ELISPOT assay used in the trial exclusively assessed donor-specific T cell responses, in the presence of a higher HLA mismatch burden, there may be a higher

TABLE 3 Adverse events (safety population) between the three study groups

	E−/SOC (*n* = 53)	E−/LI (*n* = 48)	E+ (*n* = 66)	*p* value
Any AE	53 (100)	4 (97.9)	59 (95.2)	.242
Any SAE	14 (26.4)	7 (14.6)	NA	.143
Infections				
Any infection	33 (62.3)	26 (54.2)	42 (67.7)	.347
Any viral infection	25 (47.2)	16 (33.3)	35 (56.5)	.054[a]
CMV infection	12 (22.6)	12 (25)	25 (40.3)	.079
CMV disease	4 (7.5)	1 (2.1)	4 (6.7)	.442
BKV infection	16 (30.2)	6 (12.5)	11 (16.9)	.063[b]
PVAN	5 (9.4)	0 (0)	1 (1.5)	.021[c]
Other (EBV, HSV, VZV)	5 (9.4)	1 (2.1)	6 (11.8)	.178
Any bacterial infection	19 (35.8)	19 (39.6)	15 (29.4)	.560
Any fungal infection	0 (0)	1 (2.1)	1 (2)	.580
Hematological disorders				
Hemoglobin (g/dL)	12.40 ± 4.04	11.50 ± 4.30	13.55 ± 1.86	.196
Leukocytes (1/nL)	7.62 ± 2.79	6.86 ± 2.09	7.28 ± 1.98	.355
Thrombocytes (1/nL)	229.02 ± 57.78	214.31 ± 59.47	204.11 ± 41.60	.236
Metabolic disorders				
NODAT	8 (15.1)	7 (14.6)	9 (17.6)	.903
Cholesterolemia (mmol/L)	4.86 ± 1.35	4.69 ± 0.98	4.45 ± 1.02	.450
Triglyceridemia (mmol/L)	1.86 ± 1.78	1.69 ± 0.96	1.55 ± 0.54	.766
Cardiovascular disorders				
Hypertension	43 (84.3)	40 (85.1)	39 (76.5)	.463
Cardiovascular events	3 (5.7)	3 (6.3)	5 (13.5)	.345
Cancer of any grade	3 (5.7)	4 (8.5)	5 (9.8)	.726

Abbreviations: AE, adverse event; BKV, BK virus; CMV, cytomegalovirus; E−/LI, donor-specific ELISPOT negative/low immunosuppression; E−/SOC, donor-specific ELISPOT negative/standard of care immunosuppression; E+, donor-specific ELISPOT positive; EBV, Epstein-bar virus; HSV, Herpes simplex virus; NODAT, New onset diabetes mellitus; PVAN, Polyomavirus virus nephropathy; SAE, serious adverse event; VZV, varicella-zoster virus.

Data are mean ± SD or n (%).

[a]Any viral infection: E−/SOC vs. E−/LI $p = .157$; E−/SOC vs. E+ $p = .321$; E−/LI vs. E+ $p = .016$.

[b]BKV infection: E−/SOC vs. E−/LI $p = .031$; E−/SOC vs. E+ $p = .088$; E−/LI vs. E+ $p = .516$.

[c]PVAN: E−/SOC vs. E−/LI $p = .029$; E−/SOC vs. E+ $p = .052$; E−/LI vs. E+ $p = .388$.

likelihood that patients with the same immunological alloreactive background could display a positive test against a specific donor than against others with better HLA matching.[17,31]

The CELLIMIN trial was safe, as patient and graft survival were comparable across the three different groups. However, although no differences were observed in patients remaining on protocol, E−/LI displayed the lowest kidney graft function until month 12, which could be influenced by the slightly higher TAC trough exposure and higher BPAR rates. Remarkably, a significantly lower incidence of viral infections, particularly BK viremia and PVAN, was detected only among patients receiving TAC monotherapy. These data suggest that early MMF and prednisone withdrawal leads to a lower global immunosuppressive burden.

A main limitation of the CELLIMIN trial was its premature termination due to insufficient recruitment rates, which illustrates the complexity of conducting large, prospective randomized trials using

novel biomarkers. The stringent inclusion criteria used, reducing the number of potential candidates with a more limited economical support accounted for this main drawback. Nevertheless, we could prospectively analyze an important number of patients allocated in three study groups after the biomarker intervention, thus providing unique biological and clinical information which will help designing future clinical trials further expanding on this hypothesis. We did not randomize E+ patients into LI or SOC therapy due to ethical concerns, so while we cannot rule out the possibility that E+ with a low-risk eplet mismatch score could safely receive TAC monotherapy, the higher BPAR rates among E+ than E−/SOC patients, both receiving the same immunosuppressive regimen, strongly discourages this option. Importantly, all ELISPOT assays were performed in each participating center using the same validated SOP, thus demonstrating for the first time the safety and feasibility of implementing this technology in clinical practice. Last, typing DP and DRB3/4/5 HLA loci was unfortunately not feasible, thus precluding the study of their impact on clinical outcomes. However, the consistent differences observed between groups using high-resolution HLA typing at all other class I and II locus counterbalance this constraint.

In conclusion, the results of the CELLIMIN trial strongly suggest the value of refining current immune-risk stratification by monitoring preformed T cell memory and primary alloimmune activation using the IFN-γ ELISPOT assay and HLA eplet mismatching. While the benefits of *de novo* TAC monotherapy as compared to current triple SOC therapy seem not to be supported even in low immunological risk patients, the combined risk assessment of preformed memory and *de novo* alloimmune activation seems to have the potential to help decision-making regarding immunosuppression therapy. Patients with low preformed donor-specific memory and low HLA-eplet mismatch seem to benefit from immunosuppression minimization with TAC monotherapy, which is about a quarter of first kidney transplant patients. This must be confirmed in prospective multicenter trials. In addition, new immunosuppressive approaches are warranted to increase the pool of low-risk patients, ultimately allowing safe immunosuppression minimization.

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DISCLOSURE

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

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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