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Sequential administration of anti-complement component C5 eculizumab and type-2 anti-CD20 obinutuzumab for the treatment of early antibody-mediated rejection after kidney transplantation: A proof of concept

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ABSTRACT

Kidney transplant (KT) candidates with donor-specific antibodies (DSA) exhibit exceedingly high antibody-mediated rejection (ABMR) and allograft loss rates. Currently, treatment of ABMR remains an unmet clinical need. We report the use of the anti-C5 eculizumab and the type-2 anti-CD20 obinutuzumab in two patients with early ABMR. Eculizumab (900 mg IV) led to complete inhibition of the terminal complement cascade (unremarkable AP50 and CH50 activity) and prompt stoppage of complement-dependent antibody-mediated allograft injury (clearance of intra-graft C4d and C5b-9 deposition). Despite complement inhibition, obinutuzumab (1000 mg IV) determined full and long-lasting peripheral B-cell depletion, with significant reduction in all DSA. Graft function improved, remaining stable up to three years of follow-up. No signs of active ABMR and rebound DSA were detected. Obinutuzumab B-cell depletion and inhibition of DSA production were not affected by complement blockage. Further studies are needed to confirm the potential benefit of obinutuzumab in association with complement inhibitors.

Abbreviation: KT, kidney transplant; DSA, donor-specific anti-HLA antibodies; ABMR, antibody-mediated rejection; IVIg, intravenous polyclonal human immunoglobulin; moAb, monoclonal antibody; aHUS, atypical haemolytic uremic syndrome; NITp, Nord Italia Transplant program; EDTA, ethylenediaminetetraacetic acid; SAB, single-antigen bead; MFI, mean fluorescence intensity; MAC, Membrane Attack Complex; PRA, panel-reactive antibody; CDC, complement-dependent cytotoxicity; AP50, alternative pathway 50% method; CH50, classical pathway 50% method; C4d, C4 decay product; IHC, immunohistochemistry; i, inflammation; t, tubulitis; v, endarteritis; g, glomerulitis; ptc, peritubular capillaritis; ci, cortex interstitial fibrosis; ct, cortex tubular atrophy; cv, arterial intimal fibrosis; cg, chronic glomerulopathy; ti, total cortical inflammation; i-IFTA, inflammation in scarred cortex; t-IFTA, tubulitis in tubules within scarred cortex; SCr, serum creatinine concentration; DGF, delayed graft function; dd-cfDNA, donor-derived cell-free DNA; NGS, Next Generation Sequencing; PEX, plasma exchange; IRR, infusion-related reaction; ACIP, Advisory Committee on Immunization Practices; CMV, Cytomegalovirus; FSGS, focal segmental glomerulosclerosis; PRD, primary renal disease; RRT, renal replacement therapy; PNI, Programma Nazionale Iperimmuni; KDPI, Kidney Donor Profile Index; rATG, rabbit anti-thymocyte globulin; MMF, mycophenolate mofetil; TMA, thrombotic microangiopathy; qPCR, quantitative polymerized chain reaction..

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1. Introduction

Kidney transplant (KT) candidates with previous immunization represent 15% of the patients engulfing the transplant waiting list in the Eurotransplant zone [1] and up to 25% in the United States [2]. In this group of potential recipients, those without a suitable living donor can rarely benefit from desensitization programs [3]. Therefore, they often arrive at transplant with high-level donor-specific anti-HLA antibodies (DSA) [4]. It is well known that the presence of preformed DSA at the time of transplant is a strong predictor of early antibody-mediated rejection (ABMR) and premature allograft loss due to immunological causes [5]. Although in recent years, there have been remarkable advancements in desensitization protocols [6], effective treatment of early ABMR remains an unmet clinical need [7].

Current standard of care of ABMR includes an aphaeretic technique for DSA removal and administration of low-to-high-dose intravenous polyclonal human immunoglobulin (IVIg) for DSA blockage or clearance. Some centres also advocate the use of the type-1 anti-CD20 monoclonal antibody (moAb) rituximab for B-cell depletion and down-regulation of anti-HLA antibodies production [8]. Whilst the results of anti-rejection schemes containing the anti-CD52 moAb alemtuzumab [9] or the proteasome inhibitor bortezomib [10] are overall unconvincing, data on eculizumab, a moAb targeting the complement component C5, suggest a potential short-to-mid-term benefit in patients with early ABMR [11–16]. Moreover, there is mounting interest in the type-2 anti-CD20 moAb obinutuzumab [17]. Two recent desensitization trials in KT have shown more effective peripheral and central B-cell depletion with improved transplant rates compared to IVIg and/or rituximab [18,19]. Relevantly, as demonstrated by our group in a KT recipient with atypical haemolytic uremic syndrome (aHUS) due to CFHR1/CFHR3 homozygous deletion and anti-CFH antibody, eculizumab and obinutuzumab can be sequentially administered to achieve prompt complement inhibition, profound B-cell depletion, and sustained antibody production blockage [20].

We herein describe the use of eculizumab and obinutuzumab for the treatment of early ABMR in two high-immunological-risk KT recipients, aiming to explain the rationale behind the proposed anti-rejection strategy, as much as the effects on complement function, B-cell count, DSA production, and allograft histology.

2. Materials and methods

2.1. HLA typing and anti-HLA antibodies screening

Both recipients and donors HLA typing were performed by molecular based methods. Anti-HLA antibodies were tested in the eluate from each histological specimen. Blood samples were collected, processed, and stored at the Nord Italia Transplant Program (NITp) Central Laboratory as follows. For recipients and donors, 7 mL of peripheral blood with ethylenediaminetetraacetic acid (EDTA) were retrieved to extract DNA by the EZ1 Advanced XL (Qiagen, Venlo, The Netherland). Concentration and 260/280 ratio were evaluated with NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts). Only for recipients, 7 mL of blood in tubes without anticoagulant were centrifuged at 2500 rpm/15 min, and serum was separated and transferred in 1.5 mL tubes. Sera were then filtered with centrifuge tube filter 0.22 μm and stored at −40 °C. Other 14 mL of peripheral blood with EDTA were collected and processed within 24 h from the collection. Tubes were centrifuged at 2500 rpm/15 min, plasma was then centrifuged at 10000 *g*/10 min, the supernatant transferred in 1.5 mL tubes and then frozen at −20 °C until use. DNA was isolated using the Cell3 Xtract kits (BFS Molecular SRL, Bucharest, Romania) and the concentration was evaluated with a Qubit 3.0 Fluorimeter (Thermo Fisher Scientific, Waltham, Massachusetts). Pre- and post-transplant anti-HLA antibodies were quantified using Luminex-based single-antigen bead (SAB) assays (LABScreen, One Lamba Inc., West Hills, California). Basically, the Luminex-based SAB assay is a quantifiable fluorescence test in which the purified single HLA is fixed to a given microparticle bead, enabling detection of a wide range of anti-HLA antibodies. EDTA (0.3% final) treated serum samples were first screened using LSM12 commercial kit (One Lamba Inc., West Hills, California) to identify samples negative or positive for the presence of anti-HLA antibodies. Positive sera were subsequently tested for Class-I and Class-II IgG HLA antibodies using the commercially available LABScreen SAB assay kit (SA1_4, SA2, One Lamba Inc., West Hills, California) based on single HLA molecule attached beads (either Class-I or Class-II). The test serum was analysed through the Luminex FlexMap 3D platform. The presence of specific anti-HLA antibodies was detected by the signal from phycoerythrin bound to the second anti-human IgG. The procedure was performed according to the manufacturer's instructions, and samples were analysed using Luminex xPonent 4.2 for FlexMap 3D software (Luminex Corporate, Austin, Texas) for data acquisition. Data analysis was done with HLA Fusion software (One Lamba Inc., West Hills, California). Anti-HLA serum reactivity was expressed as mean fluorescence intensity (MFI); an MFI ≥1000 was considered as positive. MFI values are a semi-quantitative measure of antibody levels. In clinical practice, preset anti-HLA antibody MFI thresholds are used to assess the need and efficacy of desensitization protocols or anti-rejection treatments, as well as to confirm the diagnosis or follow the course of ABMR. As there is no consensus in the literature (with several proposed MFI cut-off values), the choice of the MFI threshold ultimately remains a prerogative of local laboratories and transplant physicians [21].

2.2. Complement-fixing anti-HLA antibodies screening

Neat MFI values may not be able to fully predict the clinical behaviour of anti-HLA antibodies. For this reason, in line with the latest research trends, we assessed circulating DSA complement fixation abilities via solid-phase platforms, using the C1qScreen assay (One Lamba Inc., West Hills, California) [21]. The rationale behind this test is that C1q binding represents the initial step leading to the formation of the C5b-9 complex (membrane attack complex, MAC) and subsequent complement-dependent cell lysis. Accordingly, there are studies demonstrating that KT recipients with C1q-fixing anti-HLA antibodies are at increased risk of ABMR and transplant failure compared to those with non-complement binding anti-HLA antibodies [22]. Furthermore, it has been suggested that combining a complement-binding single-phase assay with pre-treatment of the serum with EDTA (see paragraph 2.1) can minimize the occurrence of false negative or falsely reduced MFI levels (so called prozone effect, caused by undetermined inhibitory agents in the serum of the recipient) [23].

2.3. Panel reactive antibody test and donor-recipient cross match

Transplant candidates exhibiting high-level preformed anti-HLA antibodies are at increased risk of hyperacute or early ABMR when receiving a kidney from a donor with the target HLA antigens. Since the publication of the seminal study by Patel and Terasaki [24], the panel-reactive antibody (PRA) has been universally accepted as a measure of pre-transplant sensitization. PRA test ultimately represents a surrogate method enabling the detection of sensitized patients, as much as the estimation of their likelihood of finding a cross match-compatible donor using a panel of normal blood donors as representative of the potential local organ donor pool [25]. Both the patients herein described were highly sensitized individuals with pre-transplant Class I and/or Class II PRA >95%.

To minimize the risk of early ABMR, it is standard practice to cross match potential donors and recipients using a cell-based or a flow cytometry cross match before transplant. In our series, donor-recipient HLA compatibility was assessed via a complement-dependent lymphocytotoxicity (CDC) cross match. This test is based on cell viability, evaluated under fluorescence microscopy by an operator after proper

staining [26]. A pre-transplant positive CDC assay is generally associated with poor allograft survival. Hence, outside the specific (and limited) field of HLA-incompatible solid organ transplantation, it is considered as a formal contraindication to KT [27].

2.4. Complement system function

Complement cascade function was monitored by serial determinations of plasma complement component C3 concentration as much as measuring AP50 and CH50. AP50 and CH50 tests assess complement haemolytic activity in plasma or serum and represent functional assays of the alternative or classical pathway of the complement system. The AP50 (alternative pathway 50% method) is based on lysis of rabbit erythrocytes in the presence of Mg^{2+} whereas the CH50 (classical pathway 50% method) requires sheep erythrocytes pre-coated with antibody in the presence of Ca^{2+} and Mg^{2+}. Mixing the erythrocytes with dilutions of patient serum allows to identify the dilution required to lyse 50% of the available erythrocytes. Both tests are sensitive to reduction, absence, or inactivity of any component of the specific complement pathway analysed and can be used for the evaluation of inhibition or consumption of complement components. These tests are adopted as indirect methods to titrate eculizumab [28,29].

2.5. Complement-mediated allograft injury

Because activation of the complement pathways produces C4 decay products (C4d) and eventually leads to the generation of MAC, we assessed intra-allograft C4d and MAC deposition in glomerular and peritubular capillaries as indicators of complement-mediated tissue injury, before and after treatment [11,28]. In particular, evaluation of C5b-9 deposition was performed using immunohistochemistry (IHC) staining with anti-C5b-9 rabbit-polyclonal antibody ab55811 (Abcam plc, Cambridge, UK). We opted for IHC over immunofluorescence (IF) for the following reasons: 1) retrospective evaluation of formalin-fixed paraffin-embedded tissues (increased occurrence of autofluorescence with IF); 2) limited tissues availability (no isotype controls or non-stained slides to rule out autofluorescence with IF); 3) single target (MAC) on tissue specimens (no need for multiplexing and/or colocalization with IF); 4) superior durability of IHC compared to IF; 5) kit availability at our institution.

2.6. Classification of allograft pathology

Clinically driven and protocol allograft biopsies were scored following the Banff 2019 classification of renal allograft pathology, the gold standard method for the diagnosis of renal transplant rejection [30]. Briefly, current Banff reporting standardization scheme requires the evaluation of several parameters (graded from 0 to 3): i, inflammation in non-scarred cortex; t, tubulitis in cortical tubules within non-scarred cortex; v, endarteritis; g, glomerulitis; ptc, peritubular capillaritis (the extent of inflammation should be classified as focal or diffuse); C4d linear staining in peritubular capillaries or medullary vasa recta on IHC; ci, interstitial fibrosis in cortex; ct, tubular atrophy in cortex; cv, arterial intimal fibrosis; cg, chronic glomerulopathy; ti, total cortical inflammation including scarred and non-scarred cortex; i-IFTA, inflammation in scarred cortex; t-IFTA, tubulitis in tubules within scarred cortex. For the diagnosis of active ABMR the following criteria must be met: 1) histologic evidence of acute tissue injury (including microvascular inflammation, arteritis, thrombotic microangiopathy, and acute tubular injury); 2) evidence of current or recent antibody interaction with vascular endothelium (linear C4d staining in peritubular capillaries or medullary vasa recta and at least moderate microvascular inflammation); 3) serologic evidence of circulating DSA. The definition of chronic active ABMR requires the concomitant presence of: 1) morphologic evidence of chronic tissue injury (such as transplant glomerulopathy, severe peritubular capillaries basement membrane multilayering, and arterial intimal fibrosis of new onset); 2) evidence of current or recent antibody interaction with vascular endothelium (linear C4d staining in peritubular capillaries or medullary vasa recta and at least moderate microvascular inflammation); 3) serologic evidence of circulating DSA. Importantly, remote DSA should not be considered for diagnosis of active or chronic active ABMR [31].

2.7. Intra-allograft immunophenotyping

To further investigate obinutuzumab B-cell depleting properties and immunomodulatory effects, as well as to provide data (possibly useful for future comparisons) regarding lymphocytes infiltration patterns before and after treatment, we assessed intra-allograft T-cell and B-cell immune phenotypes. Routinely prepared formalin-fixed paraffin-embedded blocks were sectioned from each case at 3 μm and stained with antibodies: anti-CD3 for T lymphocytes (clone F7.2.38), anti-CD20 for B lymphocytes (clone L-26), anti-CD4 (clone 4B12), anti-CD8 (clone C8/144B), and anti-FOXP3 (clone 236 A/E7) for the subpopulations of T cells, anti-CD56 (clone 123C3) for NK cells. IHC was performed using the automatic system Agilent (DAKO, Santa Clara, California). Reactions were revealed using the ultraView Universal DAB Detection Kit (Ventana Medical Systems, Tucson, Arizona), a biotin-free, multimer-based detection system, according to the manufacture's instruction.

2.8. Renal allograft function

Kidney allograft function was assessed using serum creatinine concentration (SCr), and 24-h proteinuria. Delayed graft function (DGF) was defined as the need for dialysis within seven days of transplant.

2.9. Donor-derived cell-free DNA

As a biomarker of immunologically-mediated allograft injury (in particular, ABMR), we opted for a non-invasive diagnostic method (liquid biopsy) based on circulating donor-derived cell-free DNA (dd-cfDNA) [32]. dd-cfDNA is a fragment of donor DNA released into the blood stream after the occurrence of intra-allograft cell death; it exists in free form, and it is not bound to cells. High levels of dd-cfDNA suggests ongoing allograft injury and can be detected several days before the onset of classical rejection-associated symptoms or laboratory findings such as decreased urinary output, elevated SCr, or abnormal 24-h proteinuria [33]. Proteins or contaminants-free highly pure plasma were tested. Next Generation Sequencing (NGS) library was prepared with 1–1000 ng of DNA. DNA samples were end-repaired, A-tailed, and bound to Illumina UMI adapters (Illumina Inc., San Diego, California). DNA samples were connected to 5′ phosphorylated / 3′-dA-tailed DNA fragments in both terminals. After amplification, the library was pooled and hybridized with DNA biotin-labelled probes to enrich for the 200 targeted regions of interest. Hybridized biotin-labelled probes were captured on streptavidin-coated beads; beads were then washed multiple times to remove non-targeted DNA. Targeted library DNA was quantified and then sequenced by an Illumina MiSeq (Illumina Inc., San Diego, California). Results were considered as negative for values <1%.

2.10. Plasma exchange, off-label immunosuppressants, and infections prophylaxis

Plasma exchange (PEX) sessions were carried out using a Multi-Filtrate system (Fresenius Medical Care, Bad Hamburg, Germany), with one plasma volume exchange per session and 1:1 fresh frozen plasma replacement.

Eculizumab (Soliris, Alexion Pharmaceutical, Boston, Massachusetts), obinutuzumab (Gazyvaro, Hoffman-La Roche, Basel, Switzerland), and IVIg (IG VENA, Kedrion Biopharma, Barga, Italy) were administered following manufacture's instruction. Infusion-related reactions (IRR) and possible drug-induced side effects (including

haematologic, infectious, and neoplastic complications) were meticulously recorded during the entire follow-up as required by our institutional policy regarding the use of off-label medications.

According to the Advisory Committee on Immunization Practices (ACIP) recommendations, patients exposed to eculizumab were given post-operative IV ceftriaxone 2 g for 28 days. They also received meningitis vaccination with both a tetravalent A, C, W, and Y conjugated vaccine and a multi component serogroup B vaccine (Bexsero, Novartis, Basel, Switzerland) [34]. As for standard practice in our centre, we opted for Cytomegalovirus (CMV) universal prophylaxis with oral valganciclovir and Pneumocystis jirovecii prophylaxis with oral trimethoprim/sulfamethoxazole for six months.

2.11. Treatment-associated cost

The cost of anti-rejection treatment was calculated by roughly adding up the expenses deriving from PEX (900 € per session), IVIg (15,019 € per 2 g/kg total-dose), eculizumab (20.556 € per 900 mg), obinutuzumab (4668 € per 1000 mg), or rituximab (2067 € per 375 mg). As a reference, we considered the official market price in our country, the specific price agreed between our institutional pharmacy and pharmaceutical companies, and an average Italian male subject, with a dry weight of 70 kg (**Supplementary Table 1**).

3. Case presentation #1

A 22-year-old individual with idiopathic focal segmental glomerulosclerosis (FSGS) underwent his second deceased-donor KT in February 2021. Comorbidities included systemic hypertension, hypothyroidism, and exhausted vascular access options. The onset of the primary renal disease (PRD) was recorded at the age of three, and he started renal replacement therapy (RRT) in 2009. The patient received a first deceased-donor KT in 2013. During the follow-up, he experienced recurrent FSGS and several episodes of T-cell-mediated rejection, eventually leading to allograft failure within three years of transplant. Due to repeated rituximab administrations, he also developed anti-rituximab antibodies. In 2020, the subject was enrolled in the Programma Nazionale Iperimmuni (PNI), a pilot deceased-donor allocation program for hard-to-match candidates (Class I and/or Class II PRA $\geq$95%) enabling transplant in case of preformed DSA $\geq$1000 MFI without C1q-fixing properties.

The donor was a 42-year-old subject who had died from head trauma. Ante mortem SCr was normal, and the Kidney Donor Profile Index (KDPI) was 18 [35]. The donor and the recipient were blood group compatible and had the following HLA matching: (Donor) A2,A11; B18, B35; C4,C7; DR1,DR11(5); DQA1,DQA5; DQB5(1),DQB7(3); DPA1, DPA2; DPB4,DPB13 vs. (Recipient) A2,A29; B44(12); C1,C16; DR1, DR14(6); DQA1,DQA5; DQB5(1),DQB7(3); DPB4. Pre-operative Class-I and Class-II PRA test was 95% and 55%, respectively. CDC assay was negative. Pre-transplant screening showed 61 circulating anti-HLA antibodies (median MFI, 4743; range, 1062–22,177), with preformed DSA toward B18 (3087 MFI), B35 (5415 MFI), Cw4 (1832 MFI), and DP13 (2602 MFI). The renal allograft was extra-peritoneally positioned in the iliac fossa, via a para-rectal approach. The operation was uneventful. Induction immunosuppression consisted of IV methylprednisolone (500 mg on day 0, 1, and 2) and rabbit anti-thymocyte globulin (rATG, Thymoglobulin, Genzyme, Lyon, France) 10 mg/kg total-dose. As a maintenance, we used oral LCP-tacrolimus (Envarsus, Chiesi, Parma, Italy) adjusted to achieve a blood trough level of 12–15 ng/mL during the first three months (10–12 ng/mL thereafter), mycophenolate mofetil (MMF, Myfenax, Teva, Petach Tikva, Israel) 2000 mg a day, and prednisone 20 mg a day (progressively tapered to 5 mg a day after two months). Two prophylactic PEX were carried on post-operative day 2 and 4, followed by administration of IVIg 600 mg/kg/day.

The early post-transplant phase was complicated by prolonged anuria requiring haemodialysis (DGF). LCP-tacrolimus trough level remained consistently on target, and screening for thrombotic microangiopathy (TMA) was negative. The occurrence of surgical complications was ruled out by daily Doppler-ultrasound. On day 6, moderate allograft enlargement, severe cortical oedema, reduced intraparenchymal vascularization, and disappearance of the diastolic flow in the renal artery were noticed. The patient was brought back to theatre for exploration. The kidney was viable but swollen and engorged. A biopsy was taken from the upper pole, and empirical anti-rejection treatment with IV methylprednisolone 500 mg for five days was initiated. Histology showed clear features of active ABMR with moderate diffuse peritubular capillaritis, moderate glomerulitis, and severe diffuse C4d deposition in glomerular and peritubular capillaries (Banff 2019 score: t0-i0-ti0-ptc2-v0-cv0-g2-cg0-mm0-ci0-ct0-ah0-C4d3-iIFTA0-tIFTA0; Fig. 1). MAC deposition in glomerular and peritubular capillaries was also detected (Fig. 2). Immunophenotypic analysis of intra-allograft lymphoid infiltrate demonstrated predominant CD3+ lymphocytes, with few CD20 + cells; T lymphocytes were mostly CD8+, with no CD4+ or FOXP3+ cells. NK cells immunoreactive for CD56 were also absent (Fig. 3). The diagnosis of active ABMR was confirmed by the presence of elevated preformed DSA (anti-B18, 14,226 MFI; anti-B35, 16,394 MFI; anti-Cw4, 10,279 MFI; DP13, 10,319 MFI) and the occurrence of de novo DSA (anti-A11, 2122 MFI; anti-Cw7, 2187 MFI). Remarkably, anti-B35 antibody also showed C1q-fixing properties. Aiming to rapidly block complement-dependent antibody-mediated allograft injury, on day 9, the patient was given IV eculizumab 900 mg. As assessed by post-infusion Doppler-ultrasound scan, eculizumab administration was immediately associated with a sharp reduction of the cortical oedema and a generalized improvement of the intraparenchymal blood flow. First-line ABMR treatment also included four additional PEX (day 15, 16, 18, 19) and IVIg (2 g/kg total-dose between day 11 and 19). We observed a substantial increase in urinary output and an overall reduction in DSA levels: disappearance of anti-A11, anti-B18, anti-Cw4, and anti-Cw7; decreased anti-B35 (5800 MFI) and anti-DP13 (5415 MFI). However, SCr remained elevated. Therefore, a percutaneous allograft biopsy was taken on day 21. It showed persistent signs of active ABMR, with mild diffuse peritubular capillaritis, mild glomerulitis, and severe diffuse C4d deposition in glomerular and peritubular capillaries (Banff 2019 score: t0-i0-ti0-ptc1-v0-cv0-g1-cg0-mm0-ci0-ct0-ah0-C4d3-iIFTA0-tIFTA0; Fig. 1 and Fig. 2). A change in the pattern of the intra-allograft lymphoid infiltrate was also detected, with predominant CD20+ lymphocytes, few CD3+, CD8+, and CD4+ cells, and no FOXP3+ or NK cells immunoreactive for CD56 (Fig. 4).

Despite further PEX (seven sessions between day 23 and 36), renal function did not improve and there was a rebound in several preformed DSA levels (anti-B18, 1126 MFI; anti-B35, 5965 MFI; DP13, 7990 MFI). Considering the positive response observed after eculizumab infusion (as demonstrated by serial AP50 and CH50 tests, drug-induced complement inhibition was progressively mitigated by repeated plasma substitutions) and the lack of efficacy of PEX and IVIg in controlling DSA levels, as a rescue therapy, we opted for combined complement inhibition and B-cell depletion. Rituximab was not an option because the patient had developed neutralizing antibodies following treatment for relapsing FSGS. Accordingly, an additional dose of IV eculizumab 900 mg was administered on day 37, followed by IV obinutuzumab 1000 mg on day 41. Recorded obinutuzumab IRR were nausea, vomiting, and tachycardia (105 bpm), mostly occurring during the first hour of infusion. Despite effective complement inhibition (erased AP50 and CH50 activity), obinutuzumab led to complete peripheral CD20+ B-cell depletion within 24 h of infusion. A substantial reduction in all DSA levels was achieved in the next few weeks (undetectable anti-A11, anti-B18, anti-Cw4, and anti-Cw7; decreased anti-B35 and anti-DP13). On post-transplant day 44, the patient was discharged with excellent urinary output and satisfactory renal function (SCr, 2.1 mg/dL).

Protocol histology, obtained nine months later, demonstrated mild glomerulitis and mild focal peritubular capillaritis, with absent C4d deposition in peritubular capillaries (Banff 2019 score: t0-i0-ti0-ptc1-

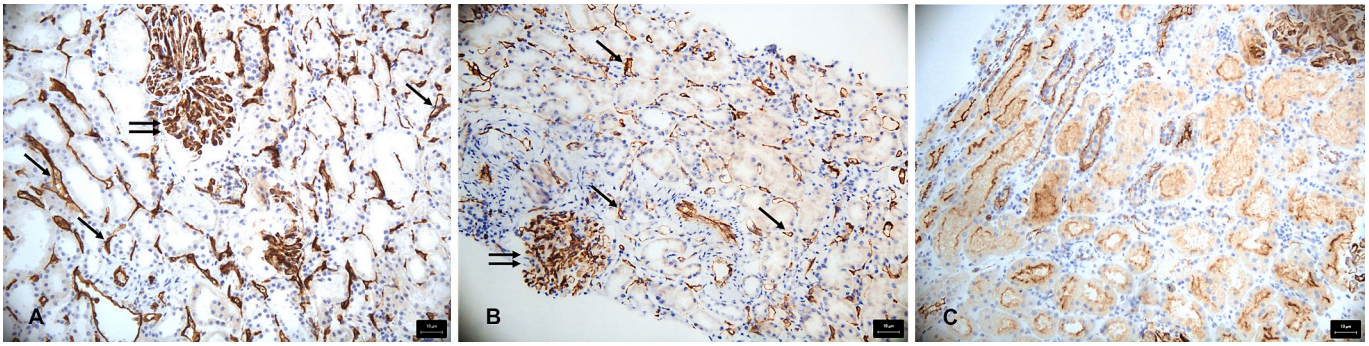

Fig. 1. Case #1: C4d IHC staining on kidney allograft biopsies obtained on post-operative day 6 **(A)**, post-operative day 21 **(B)**, and post-operative day 240 **(C)**. After eculizumab and obinutuzumab administration, we observed progressive clearance of C4d deposition in peritubular (single arrows) and glomerular (double arrows) capillaries.

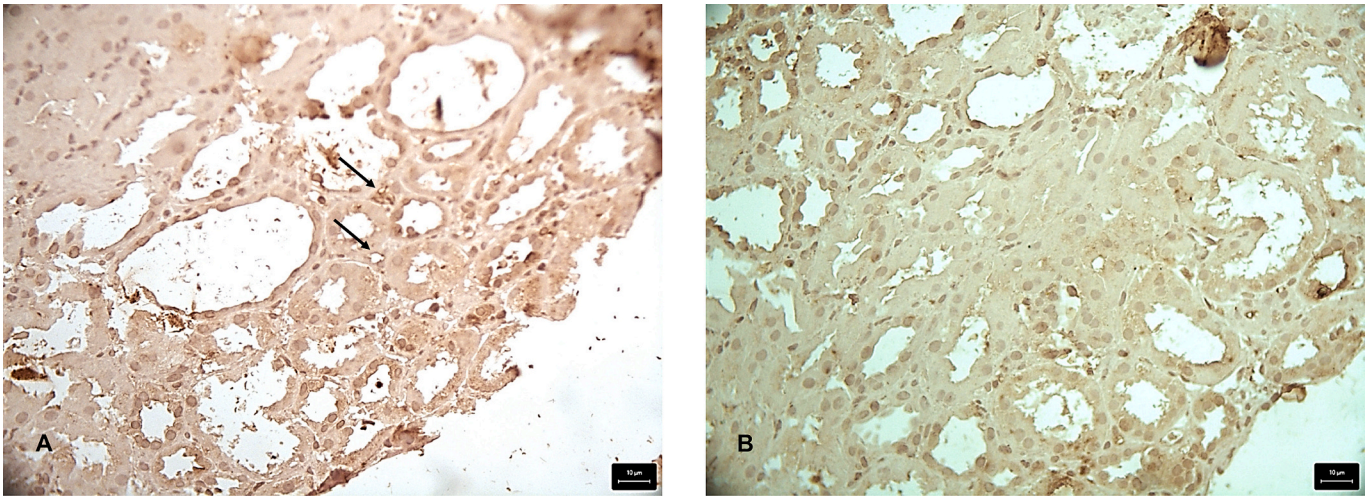

Fig. 2. Case #1: C5b-9 IHC staining (Anti-C5b-9 rabbit-polyclonal antibody ab55811, Abcam plc, Cambridge, UK) on kidney allograft biopsies obtained on post-operative day 21 **(A)** and post-operative day 240 **(B)**. After eculizumab and obinutuzumab administration, we observed clearance of C5b-9 deposition (arrows) in glomerular and peritubular capillaries.

v0-cv0-g1-cg0-mm0-ci0-ct0-ah0-C4d0-iIFTA0-tIFTA0; Fig. 1). MAC staining remained persistently negative (Fig. 2). A further reduction in anti-B35 and anti-DP13 levels (both <2000 MFI) was also noticed. Three years after transplant, the recipient is doing well, with excellent allograft function (SCr, 1.3 mg/dL; 24-h proteinuria, 0.08 g/L), normal white blood cell (9.9 cell $\times$ 10^9/L), lymphocyte (1.2 cell $\times$ 10^9/L), platelet (239 cell $\times$ 10^9/L), and red blood cell (4.3 cell $\times$ 10^{12}/L) counts, restored complement activity (CH50, 129%; AP50, 102%), sustained B-cell depletion (peripheral CD20+ cell <1%), and negative Polyomavirus BK plasma quantitative polymerized chain reaction (qPCR). At last screening, there were no de novo DSA. All preformed DSA remained undetectable, excluding low-level anti-DP13 (1700 MFI). Results from liquid biopsy (dd-cfDNA) were also reassuring. Overall, complications possibly related to obinutuzumab included a foodborne infection caused by *Campylobacter jejuni* (requiring hospitalization, supportive care, and antibiotic treatment), few episodes of asymptomatic CMV viraemia (treated with valganciclovir), an asymptomatic positivity for SARS-CoV-2, and intermittent leukopenia (managed with reduction or temporary suspension of MMF). Immunosuppression protocol and main treatment-related outcomes are summarized in Fig. 5, Fig. 6, and Fig. 7. Overall treatment-associated cost (as per national market price) was 70.697 € (PEX, 9900 €; IVIg, 15,019 €; eculizumab, 41,110 €; obinutuzumab, 4668 €).

4. Case presentation #2

A 50-year-old patient with idiopathic FSGS underwent his third KT in April 2021. Comorbidities included systemic hypertension, IgA monoclonal gammopathy of undetermined significance, and multifactorial anaemia. The onset of the PRD was recorded at the age of 18, and RRT was started in 1994. One year later, he received a kidney from his mother. The post-transplant course was complicated by recurrent FSGS, eventually leading to allograft loss in 2002. After three years, the patient underwent a second KT, also complicated by relapsing FSGS and premature allograft failure. In 2021, he was enrolled into the PNI.

The donor was a 67-year-old subject who had died from intracranial haemorrhage. Renal function was normal, with a KDPI of 94. The donor and the recipient were blood group compatible and had the following HLA matching: (Donor) A2,A3; B8,B65(14); C7,C8; DR7, DR17(3); DQA1*02,*05; DQB1*02; DPA1*02; DPB1*09,*17 vs. (Recipient) A2, A30; B13,B41; DR4,DR7; DQA1*02,*03; DQB1*02,*04. Pre-operative Class-I and Class-II PRA test was 25% and 100%, respectively. CDC assay was negative. Pre-operative assessment showed 19 circulating anti-HLA antibodies (median MFI, 2210; range, 1098–22,897), with preformed DSA toward DP9 (DPB1*09:01, 2232 MFI), DP17 (DPB1*17:01, 1982 MFI), DQA1*05:01 (2210 MFI), and DR17 (DRB1*03:01, 1715 MFI). The transplant operation was performed uneventfully. Aiming to prevent early ABMR (as observed in the previous case), we administered IV eculizumab 900 mg immediately before

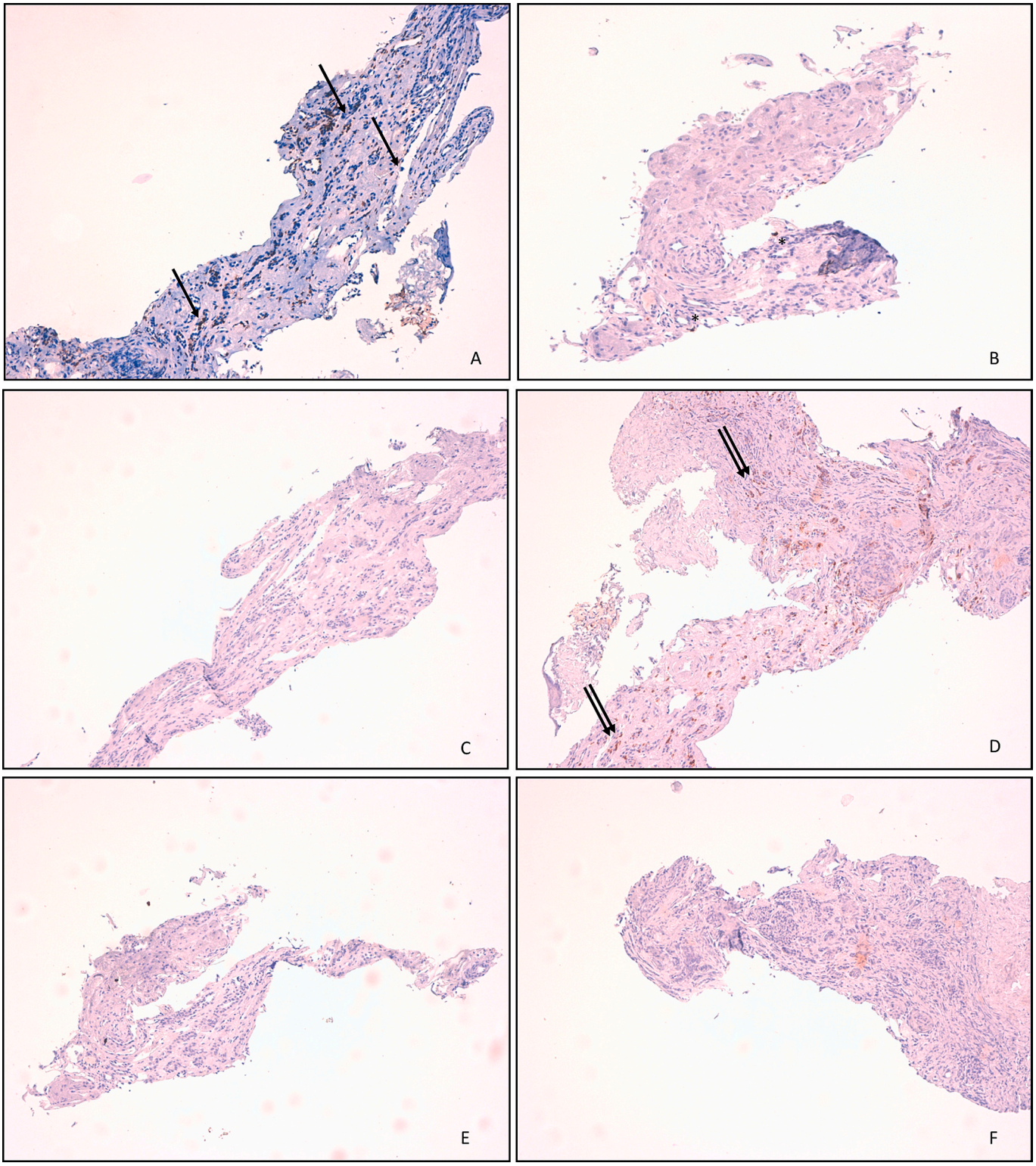

Fig. 3. Case #1: Immunophenotypic profile of lymphoid infiltrate in kidney allograft biopsy obtained on post-operative day 6, showing predominantly CD3+ lymphocytes (single arrows) **(A)**, with few CD20+ B cells (asterisks) **(B)**. T lymphocytes were mostly CD8+ (double arrows) **(D)**, with no CD4+ cells **(C)**. No NK cells immunoreactive for CD56 **(E)** or FOXP3+ cells **(F)** were detected. Original magnification: 100×.

surgery. Immunosuppression also included IV methylprednisolone (500 mg for three days), rATG (5 mg/kg total-dose from day 0 to day 4), oral standard-release tacrolimus (Adoport®, Sandoz International GmbH, Basel, Switzerland) adjusted to achieve a trough level of 12–15 ng/mL during the first month (10–12 ng/mL thereafter), MMF 2000 mg a day, and prednisone 20 mg a day (progressively tapered to 5 mg a day after two months of follow-up). Four prophylactic PEX were performed on day 4, 6, 8, and 12, followed by infusion of IVIg 2 g/kg total-dose (between day 5 and 12).

The early post-operative course was characterized by oliguria requiring haemodialysis (DGF). During the first two weeks, the urinary output progressively increased (up to 2000 mL/24 h by day 7), but renal

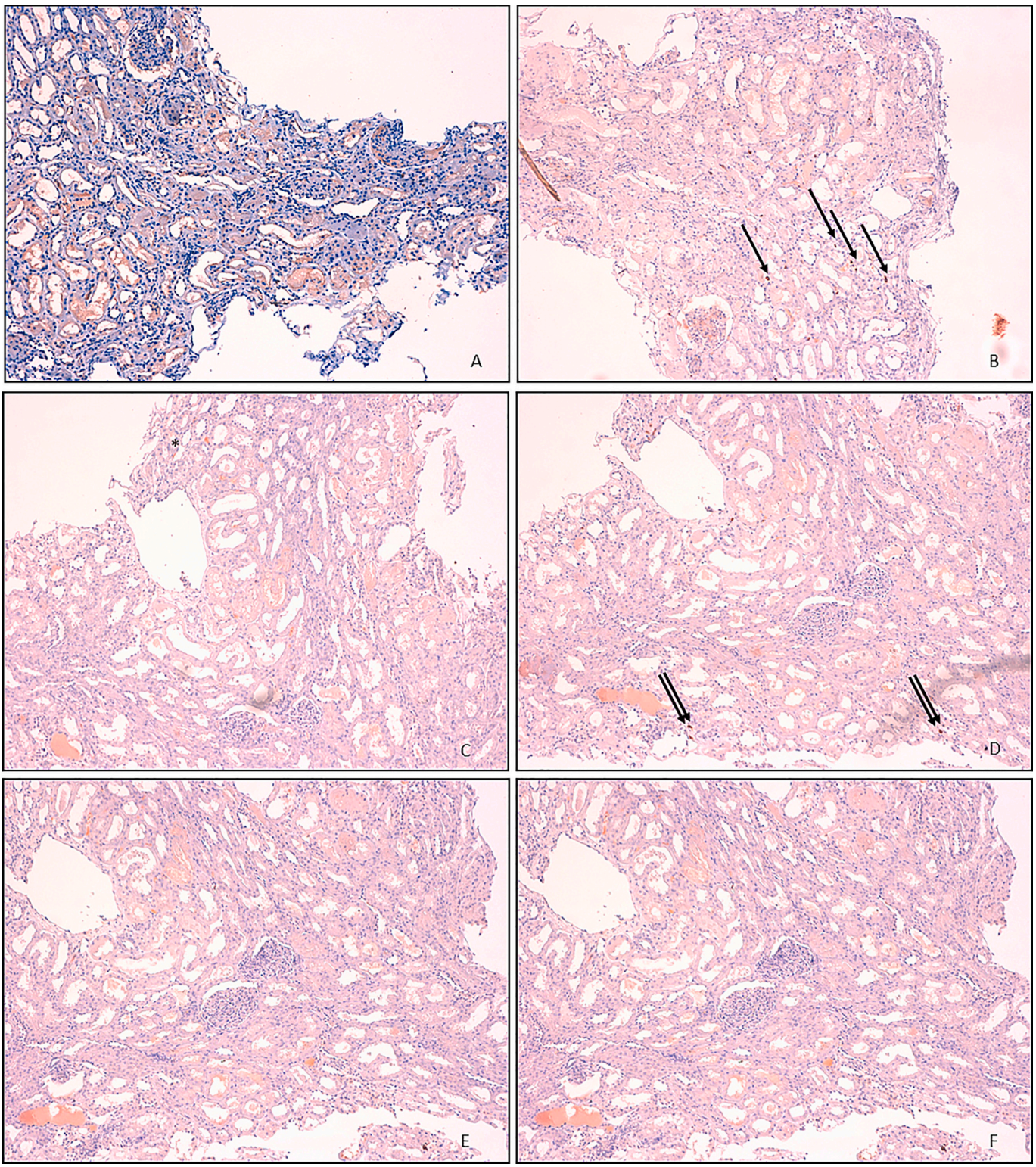

Fig. 4. Case #1: Immunophenotypic profile of lymphoid infiltrate in kidney allograft biopsy obtained on post-operative day 21, showing predominantly CD20+ lymphocytes (single arrows) **(B)**,with few CD3+ T cells **(A)**, CD8+ (double arrows) **(D)**, and CD4+ cells (asterisks) **(C)**. No NK cells immunoreactive for CD56 **(E)** or FOXP3+ cells **(F)** were detected. Original magnification: 100×.

function did not improve as expected (SCr >3.5 mg/dL). Tacrolimus levels were on target and markers of TMA were negative. A Doppler-ultrasound showed mild cortical oedema and elevated cortical resistivity index. Despite PEX and IVIg, there was a progressive increase in several preformed DSA levels (anti-DP9, 19,519 MFI; anti-DP17, 18,809 MFI; and anti-DR17, 5981 MFI). Therefore, on day 13, we performed a transplant biopsy. Pathology showed mild tubulitis, mild inflammation in the non-scarred cortex, mild total cortical inflammation, and signs of active ABMR, including mild glomerulitis, moderate diffuse peritubular capillaritis, and severe diffuse C4d deposition in glomerular and peritubular capillaries (Banff 2019 score: t1-i1-ti1-ptc2-v0-cv2-g1-cg0-mm0-ci0-ct0-ah0-C4d3-pvx-iIFTA0-tIFTA0; Fig. 8). MAC staining in

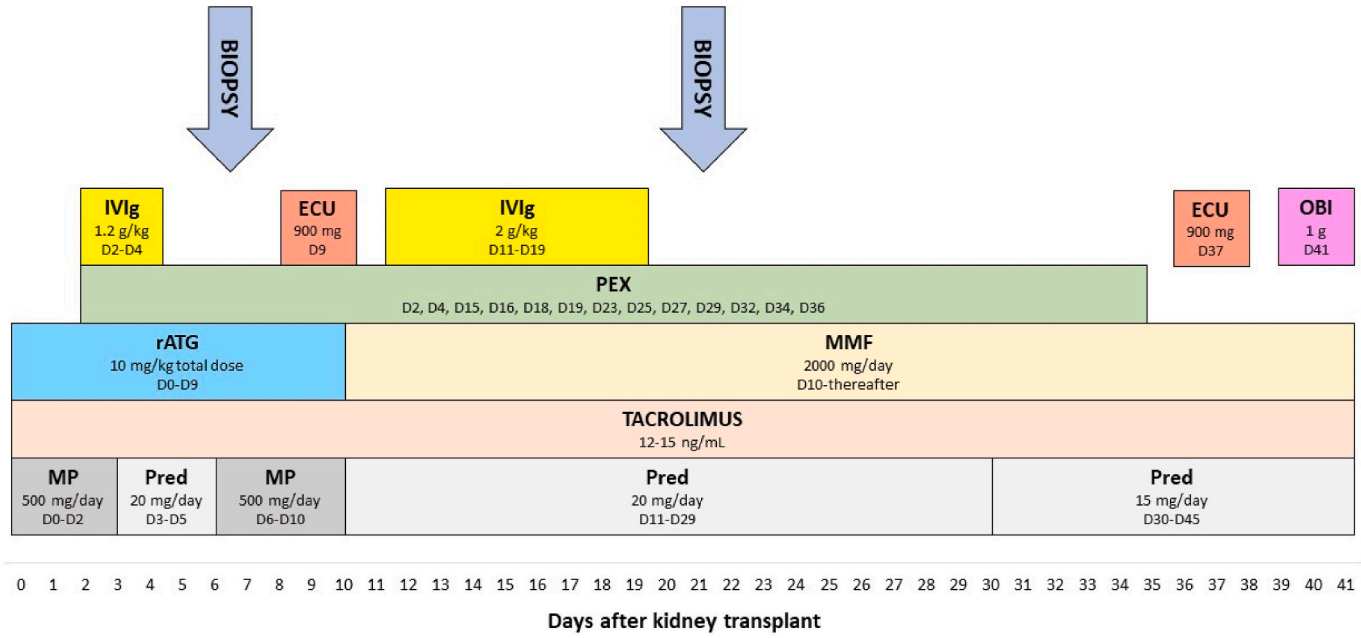

Fig. 5. Case #1: induction, maintenance, and anti-rejection immunosuppression (MP, methylprednisolone; Pred, prednisone; rATG, rabbit anti-thymocyte globulin; PEX, plasma exchange; IVIg, intravenous polyclonal human immunoglobulin; ECU, eculizumab; OBI, obinutuzumab; TAC, tacrolimus; MMF, mycophenolate mofetil).

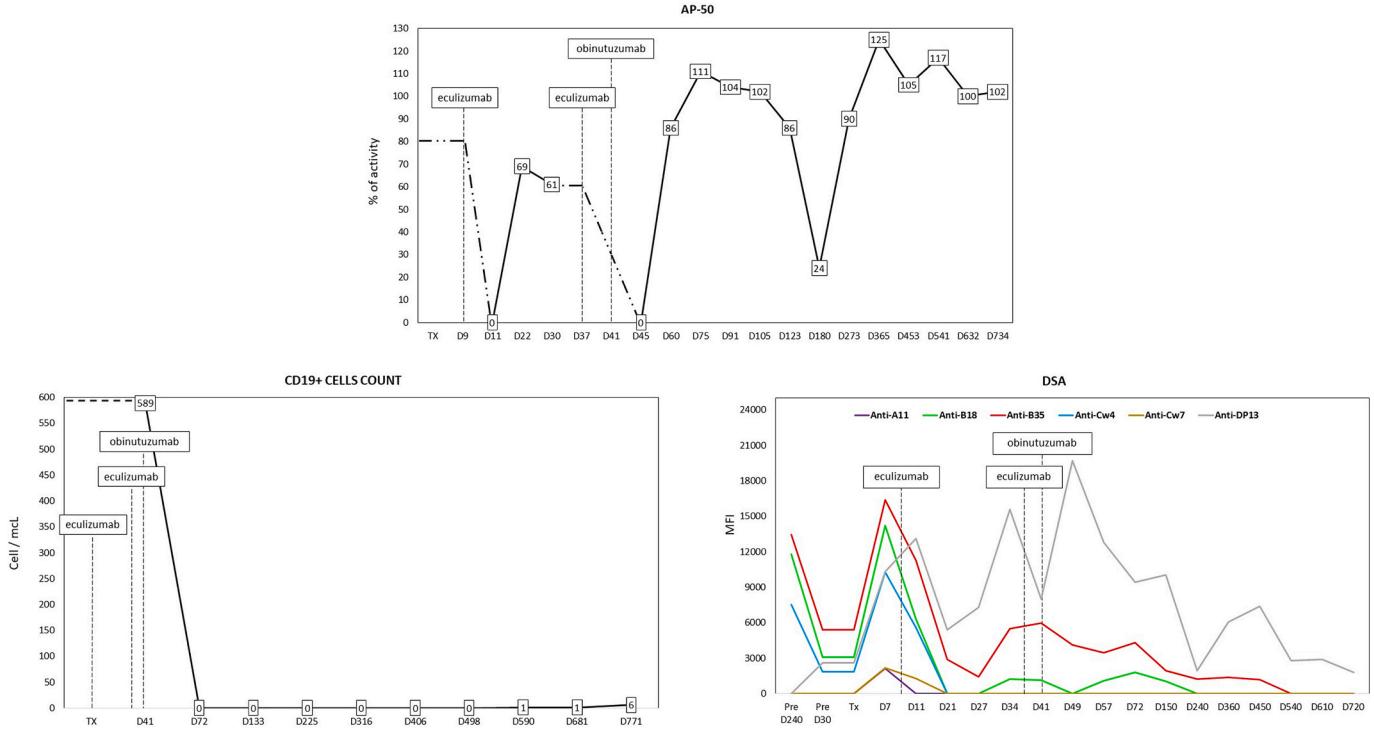

Fig. 6. Case #1: effects of eculizumab and obinutuzumab on AP50% activity **(A)**, peripheral CD19+ cells count **(B)**, and donor-specific anti-HLA antibodies (DSA) levels **(C)** over time.

glomerular and peritubular capillaries was also positive (Fig. 9). Immunophenotypic analysis of intra-allograft lymphoid infiltrate demonstrated mixed CD3+ T lymphocytes and CD20+ B cells. T cells were mostly CD8+, with no CD4+, or FOXP3+. There were no NK cells immunoreactive for CD56 (Fig. 10).

First-line treatment included IV methylprednisolone 500 mg for four days, additional PEX (on day 14, 15, 16, and 17), and IVIg (2 g/kg total-dose on day 15, 16, and 17). As DSA remained consistently detectable (anti-DP9, 1539 MFI; anti-DP17, 1259 MFI; anti-DR17, 1468 MFI) and markers of complement function (AP50 and CH50) had returned within normal range (likely due to plasma substitutions), on day 18, we administered another shot of IV eculizumab 900 mg to halt complement-dependent antibody-mediated allograft injury and prevent future damage caused by rebound DSA. Three days later, IV obinutuzumab 1000 mg was also given, aiming to persistently block DSA production. Recorded obinutuzumab IRR were nausea and tachycardia (110 bpm).

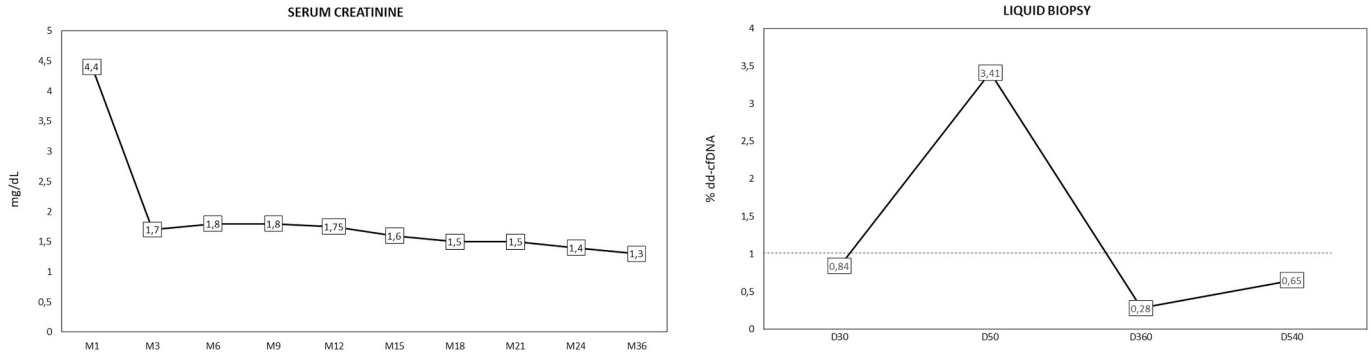

Fig. 7. Case #1: Serum creatinine concentration **(A)** and liquid biopsy (circulating donor-derived cell-free DNA, dd-cfDNA) results **(B)** over time.

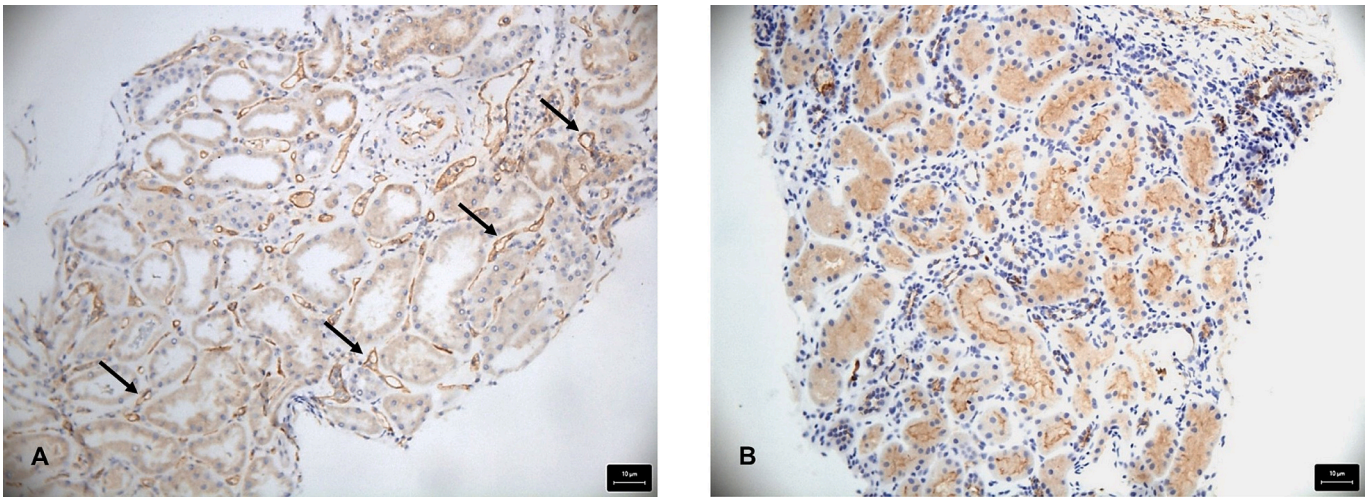

Fig. 8. Case #2: C4d IHC staining on kidney allograft biopsies obtained on post-operative day 13 **(A)** and post-operative day 180 **(B)**. After eculizumab and obinutuzumab administration, we observed complete clearance of C4d deposition (arrows) in glomerular and peritubular capillaries.

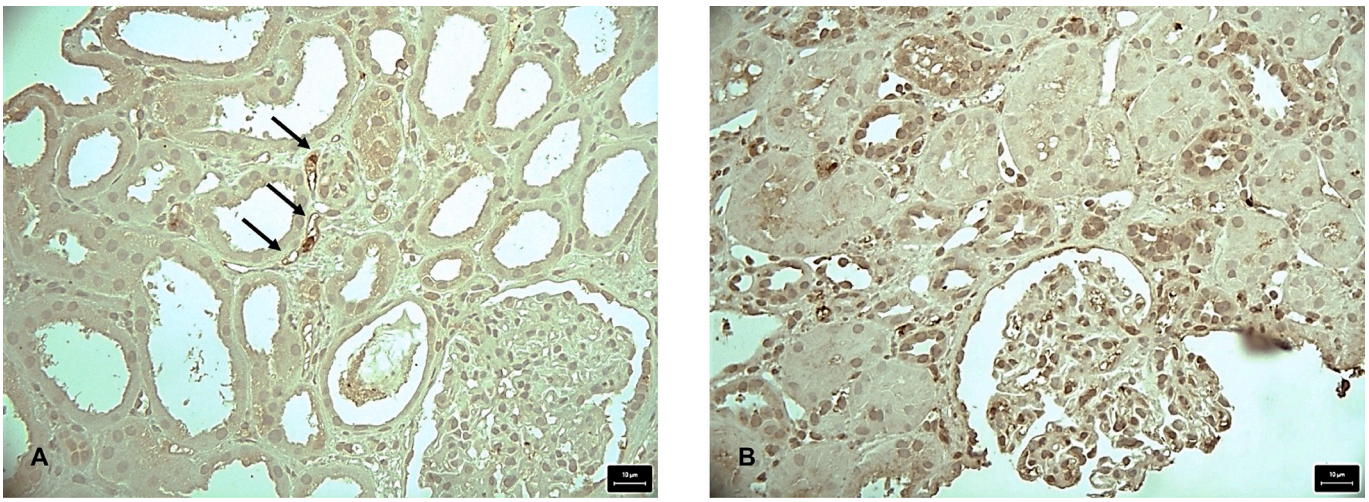

Fig. 9. Case #2: C5b-9 IHC staining (Anti-C5b-9 rabbit-polyclonal antibody ab55811, Abcam plc, Cambridge, UK) on kidney allograft biopsies obtained on post-operative day 13 **(A)** and post-operative day 180 **(B)**. After eculizumab and obinutuzumab administration, we observed complete clearance of C5b-9 deposition (arrows) in glomerular and peritubular capillaries.

Although the terminal complement cascade had been effectively inhibited by eculizumab (as assessed by AP50 and CH50 tests), full peripheral B-cell depletion and disappearance of all circulating DSA (anti-DP9, anti-DP17, and anti-DR17) was rapidly achieved. The patient was discharged on day 28, with satisfactory renal function (SCr, 2.5 mg/dL).

No features of active ABMR, residual microvascular inflammation, or complement-dependent tissue injury were detected by the protocol transplant biopsy obtained six months later (Banff 2019 score: t0-i0-ti0-ptc0-v0-cv1-g0-cg0-mm0-ci0-ct0-ah1-C4d0-pvx-iIFTA0-tIFTA0; Fig. 8 and Fig. 9). Immunophenotyping showed predominant CD3+

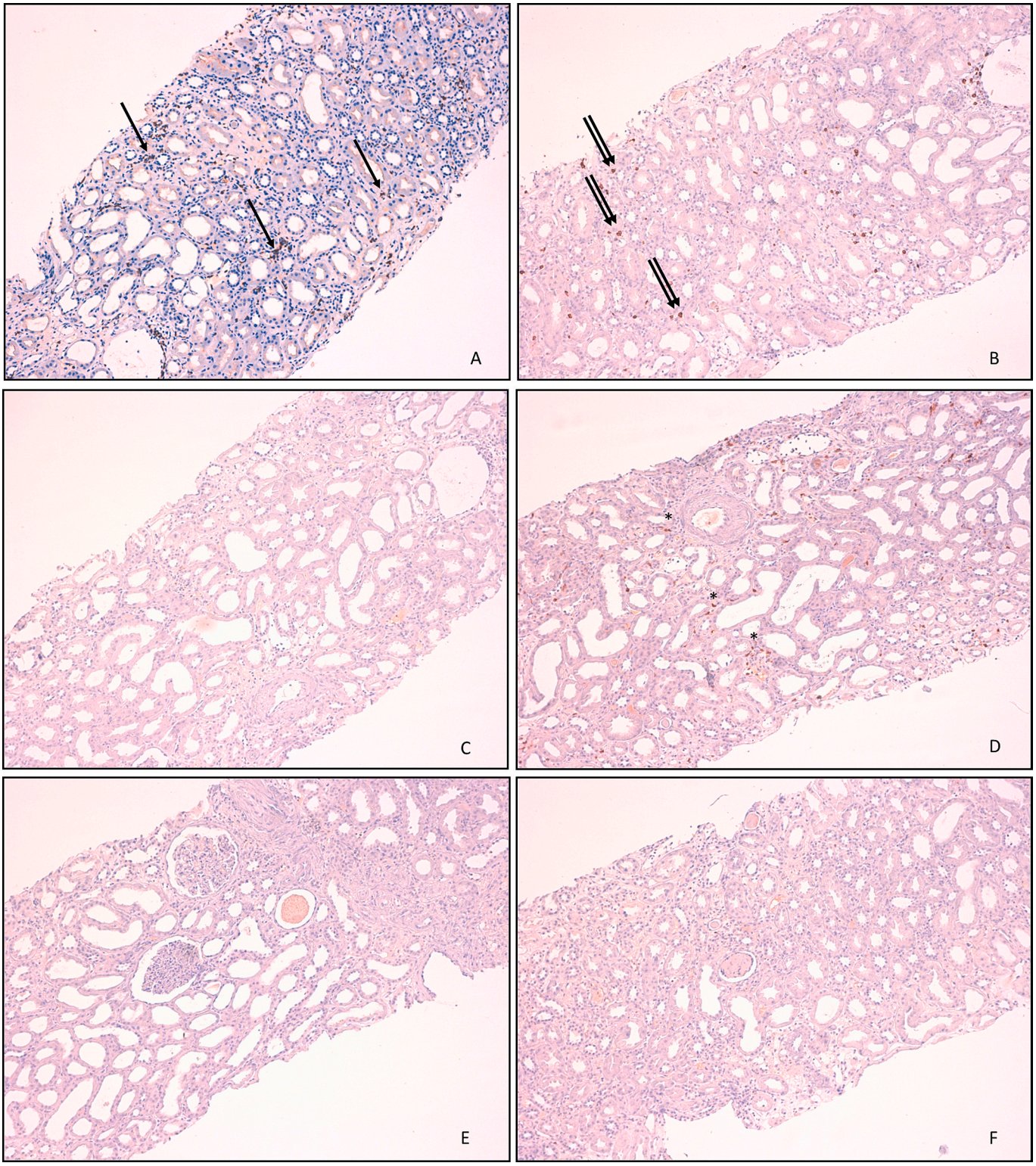

Fig. 10. Case #2: Immunophenotypic profile of lymphoid infiltrate in kidney allograft biopsy obtained on post-operative day 13, showing mixed CD3+ T lymphocytes (single arrows) **(A)** and CD20+ B lymphocytes (double arrows) **(B)**. There were no CD4+ cells **(C)** and T lymphocytes were mostly CD8+ (asterisks) **(D)**. No NK cells immunoreactive for CD56 **(E)** or FOXP3+ cells **(F)** were identified. Original magnification: 100×.

lymphocytes, with few CD20+ B cells. T lymphocytes were mostly CD8+, with no CD4+ or FOXP3+ cells. NK cells immunoreactive for CD56 were also absent (Fig. 11). Almost three years after transplant, the recipient is doing well, with good transplant function (SCr, 1.9 mg/dL; 24-h proteinuria, 0.07 g/L), normal white blood cell (5.2 cell × 10^9/L) and platelet (152 cell × 10^9/L) counts, mild anaemia (red blood cell count, 3.8 cell × 10^{12}/L), mild lymphocytopenia (0.85 cell × 10^9/L), restored complement activity (CH50, 119%; AP50, 105%), sustained B-cell depletion (peripheral CD20+ cell <1%), and negative Polyomavirus BK plasma qPCR. During the follow up, no rebound DSA or de novo DSA were identified. Screening for immunologically mediated allograft injury (circulating dd-cfDNA) remained persistently negative.

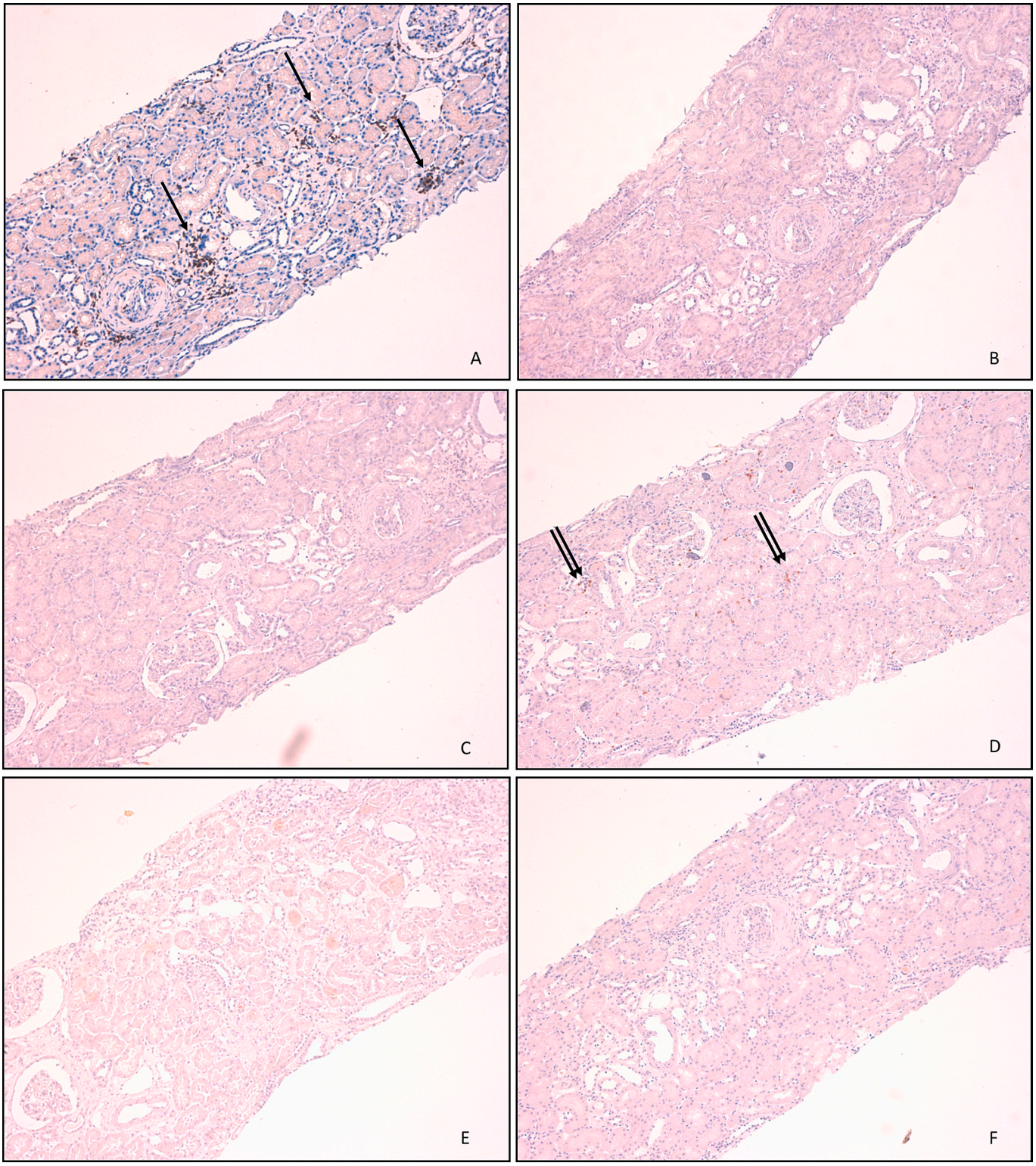

Fig. 11. Case #2: Immunophenotypic profile of lymphoid infiltrate in kidney allograft biopsy obtained on post-operative day 180, showing predominantly CD3+ T lymphocytes (single arrows) **(A),** with no B cells **(B)**. There were no CD4+ T cells **(C)** and T lymphocytes were mostly CD8+ (double arrows) **(D)**. No NK cells immunoreactive for CD56 **(E)** or FOXP3+ cells **(F)** were detected. Original magnification: 100×.

Complications possibly related to obinutuzumab included an episode of asymptomatic CMV viraemia (treated with valganciclovir), mild symptomatic SARS-CoV2 infection (managed with temporary MMF withdrawal), delayed organizing pneumonia (treated with hospitalization, steroid, and IV antibiotics), and intermittent leukopenia (requiring reduction of MMF). Immunosuppression protocol and main treatment-related outcomes are summarized in Fig. 12, Fig. 13, and Fig. 14. Overall treatment-associated cost (as per national market price) was 43,842 € (PEX, 3600 €; IVIg, 15,019 €; eculizumab, 20,555 €; obinutuzumab, 4668 €).

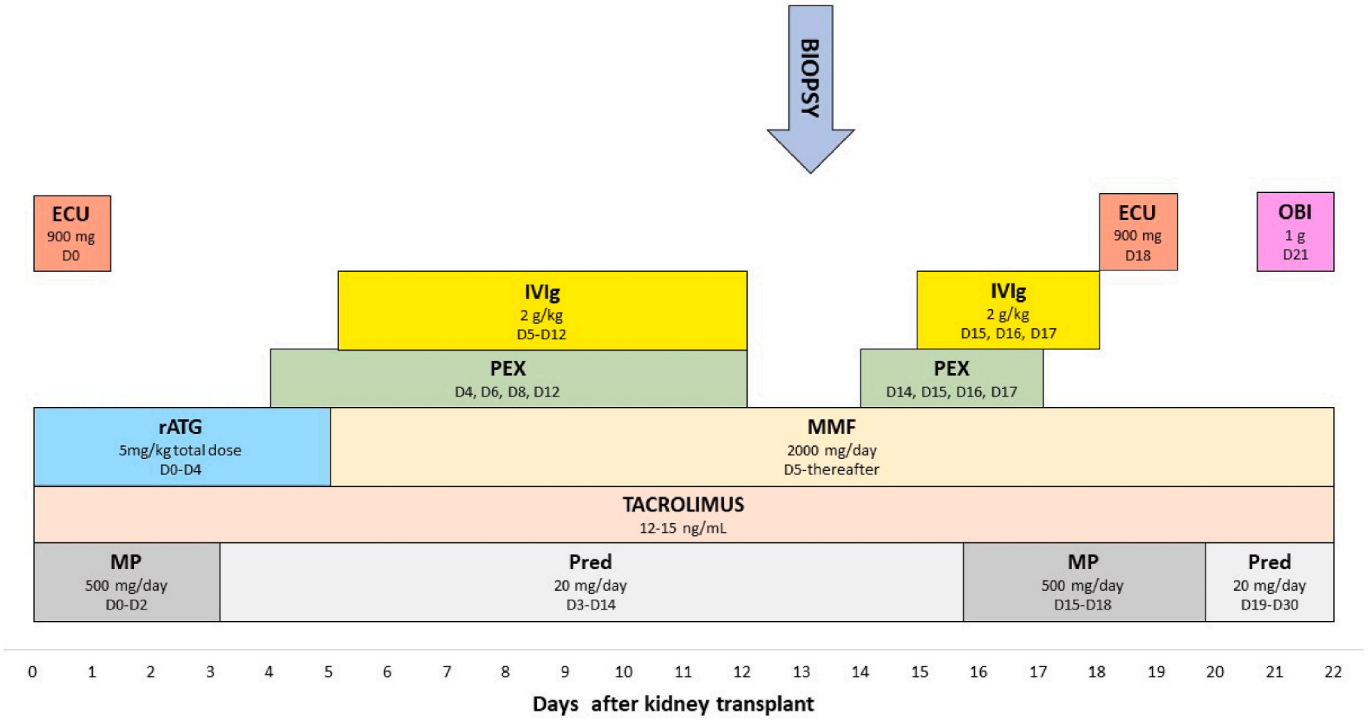

Fig. 12. Case #2: induction, maintenance, and anti-rejection immunosuppression (MP, methylprednisolone; Pred, prednisone; rATG, rabbit anti-thymocyte globulin; PEX, plasma exchange; IVIg, intravenous human polyclonal immunoglobulin; ECU, eculizumab; OBI, obinutuzumab; TAC, tacrolimus; MMF, mycophenolate mofetil).

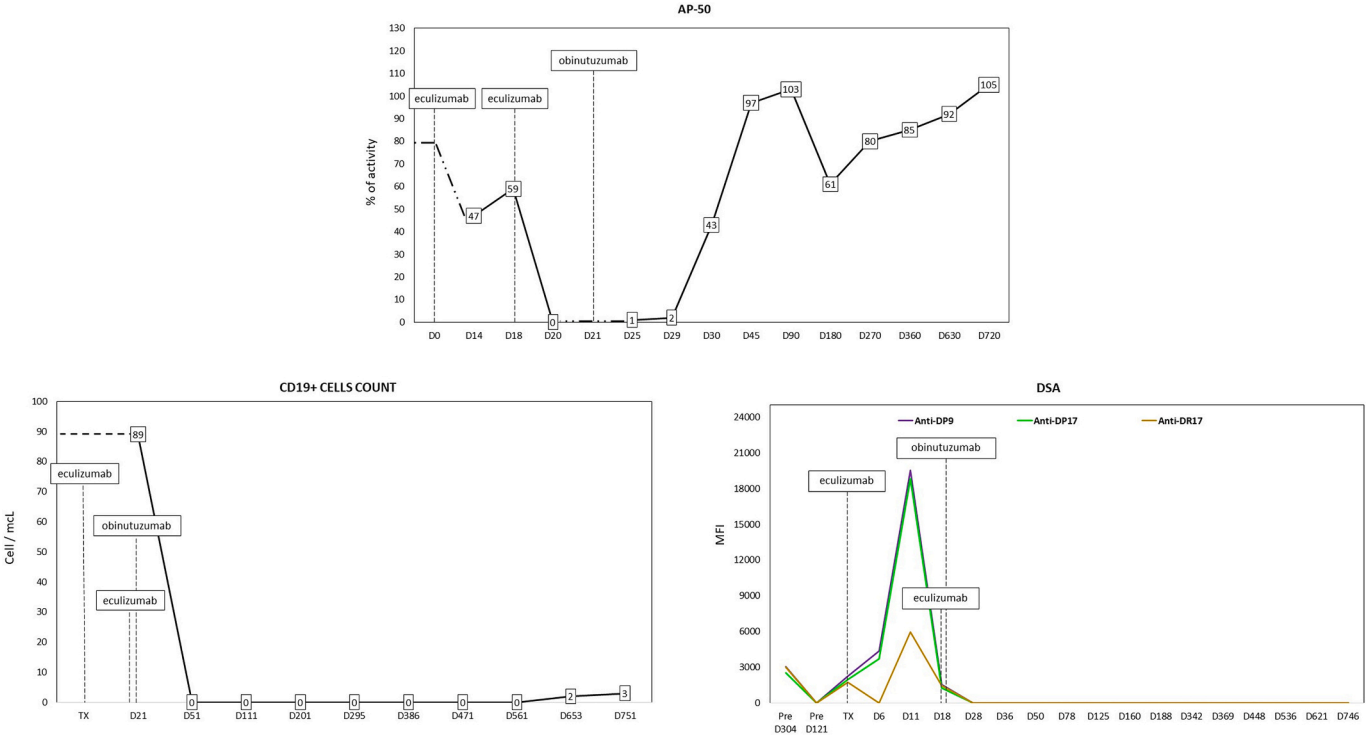

Fig. 13. Case #2: effects of eculizumab and obinutuzumab on AP50% of activity **(A)**, peripheral CD19+ cells count **(B)**, and donor-specific anti-HLA antibodies (DSA) levels **(C)** over time.

5. Discussion

This is the first experience with the combined use of eculizumab and obinutuzumab in the setting of ABMR. In both the highly sensitized KT recipients treated, we showed complete reversal of complement-dependent antibody-mediated allograft injury (C4d and MAC deposition in glomerular and peritubular capillaries), reduction of transplant microvascular inflammation (glomerulitis and/or peritubular capillaritis), and effective prevention of early-to-mid-term rebound immunological damage caused by anti-HLA antibodies, achieving prompt

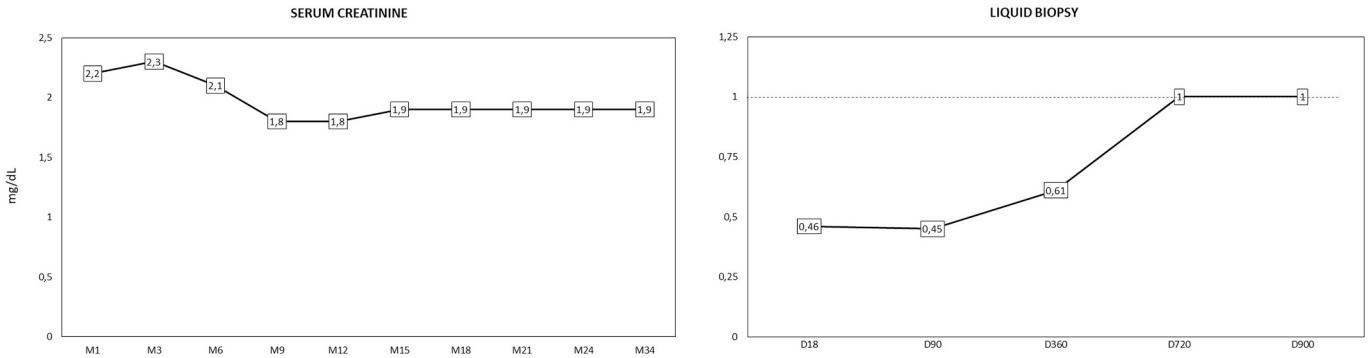

Fig. 14. Case #2: Serum creatinine concentration **(A)** and liquid biopsy (circulating donor-derived cell-free DNA, dd-cfDNA) results **(B)** over time.

complement cascade blockage, sustained peripheral B-cell depletion, and prolonged inhibition of preformed or de novo DSA production (including Class-II). After eculizumab and obinutuzumab administration, transplant function rapidly improved, remaining stable up to three years, with reassuring findings on protocol histology and liquid biopsy (circulating dd-cfDNA). Importantly, no severe IRR, major drug-induced adverse events, life-threatening infectious complications, or malignancies were recorded during the follow-up. Overall, the cost of our anti-rejection scheme was higher than the estimate for the standard of care (i.e., PEX, IVIg, and rituximab). However, the financial burden of the proposed protocol could be outweighed by the theoretical savings generated by prolonged allograft survival compared to dialysis [36].

ABMR can be observed in up to 50% of highly sensitized KT recipients, particularly in the initial post-transplant course [37,38]. Most early ABMR episodes are associated with high-level preformed DSA, but de novo DSA may also contribute [38,39]. Despite the lack of solid evidence, apheresis (namely, plasmapheresis, PEX, or immunoadsorption) and IVIg represent the preferred treatment of ABMR, with rituximab often adopted as an adjuvant agent in case of severe or refractory forms of rejection [40,41]. The aim of this multimodality strategy is to remove circulating DSA (apheresis), favor DSA inactivation or clearance (IVIg), inhibit B-cell and T-cell antigen-specific response (rituximab and IVIg), and induce DSA production blockage via B-lymphocyte depletion (rituximab). However, the vast majority of ABMR episodes are caused by DSA produced by memory B-cells, which remain unaffected by rituximab [42]. Furthermore, it has been demonstrated that DSA-induced allograft injury is greatly dependent on complement activation, another missed target of standard anti-rejection protocols [43,44].

Eculizumab is the cornerstone of aHUS management due to its rapid and effective mechanism of action, resulting in complete inhibition of the terminal complement cascade [45]. Three clinical trials evaluating eculizumab with different induction immunosuppressive schemes and aphaeretic techniques for the prevention of ABMR in high-risk KT candidates have found a reduced incidence of early ABMR, with acceptable complication rates [12,14,15]. Also, eculizumab administration in patients with ABMR has increasingly been reported with interesting short-term results [11,16]. Following this trend, we decided to use eculizumab to halt ongoing complement-dependent antibody-mediated allograft injury, as well as to prevent possible damage caused by preformed DSA not effectively removed by PEX, rebound DSA, or de novo DSA. In our experience, eculizumab use was rapidly associated with reduced cortical oedema, improved renal intra-parenchymal blood flow, and increased urinary output. Furthermore, after eculizumab infusion, allograft histology demonstrated a significant amelioration of cortical microvascular inflammation and complete disappearance of C4d and MAC deposition in glomerular and peritubular capillaries [11,46].

Between 2009 and 2023, at least 22 English-edited papers mentioning the use of eculizumab for the treatment of active ABMR have been published (Table 1). In most cases, eculizumab was administered as a rescue therapy or as a part of complex multimodality schemes containing apheresis, IVIg, and/or rituximab. Overall, we could retrieve information regarding 53 patients who had experienced ABMR within two weeks of transplant (as described in the present series). Extended follow-up data (>1 year) were rarely reported. Nevertheless, treatment-related outcomes included: ABMR resolution ($n = 33$), allograft loss ($n = 10$), and development of chronic active ABMR ($n = 4$). Reviewing all these studies, it sounds plausible to say that the efficacy of eculizumab-based treatments is higher when ABMR is diagnosed in the very early post-transplant phase and recipients are treated shortly after the occurrence of rejection. The hypothesis that early ABMR is more responsive to eculizumab than late ABMR, is supported by recent evidence showing substantial differences in term of clinical features, allograft histology, anti-HLA antibody characteristics, and long-term prognosis [47]. Also, current evidence suggests that eculizumab should be used as soon as possible, aiming to stop complement-dependent antibody-mediated allograft injury before it may cause irreversible tissue damage or trigger the development of chronic active ABMR [48,49]. Finally, considering the high rates of allograft loss or chronic active ABMR recorded after early ABMR episodes, it appears as the identification of more effective B-cell depleting agents might represent a key factor for the long-term survival of the transplant [50]. As for previous case reports or case series describing anti-rejection treatments in patients who had been exposed to complex immunosuppressive regimens [51], it can be argued that the previous use of PEX and/or IVIg might represent a potential source of bias, making it challenging to assess the specific impact of eculizumab and/or obinutuzumab on transplant-related outcomes. However, the primary aim of the present report was to describe the short- and mid-term effects of obinutuzumab on peripheral B-cell count, DSA levels, allograft histology, and patient safety in the unexplored setting of ABMR and eculizumab-induced complement inhibition. In this regard, our work should be interpreted as a proof of concept rather than a conclusive analysis on the efficacy and safety of eculizumab and obinutuzumab for the treatment of ABMR. Furthermore, when evaluating the clinical benefit arising from obinutuzumab, it should be noticed that, at the time of administration, our patients had clear histological features of active ABMR (glomerulitis, diffuse peritubular capillaritis, and severe diffuse C4d deposition), signs of intra-allograft complement-mediated tissue injury (C4d and MAC deposition), and several circulating preformed or de novo DSA, despite repeated PEX, high-dose IVIg, and eculizumab [39,46,52,53]. Since most of the studies on ABMR describe a multimodality approach (Table 1), next trials should focus on the upfront use of eculizumab and/or obinutuzumab, avoiding aphaeresis. In fact, apart from the theoretical risk of bias, aphaeretic techniques can impair the effectiveness of previously administered therapeutic agents (in our case, eculizumab), and are often associated with logistic challenges, extended cold ischemia time, adverse events, and increased costs [54]. On the contrary, we believe that including IVIg in future ABMR prophylaxis or treatment protocols might prove beneficial as they favor T-cell and B-cell immunomodulation as much as DSA inactivation and clearance [55], while

Table 1
Most relevant studies on eculizumab and/or obinutuzumab use for the treatment of anti-HLA antibody-mediated rejection after kidney transplantation.

Reference (Year)	KT (n)	Immunosuppression	ABMR timing	ABMR treatment	FU	Outcomes
Locke 2009	1	Pre-op: PP + IVIg Post-op: DAC + PP + IVIg	8d	PP + IVIg ECU + RTX + IVIg PP + IVIg	4 m	ABMR resolution
Chehade 2015	1	Pre-op: IVIg Intra-op: BAX	5d	PP + ECU ATG PP + ECU IVIg	9 m	ABMR resolution
Schwotzer 2020	1	Intra-op: ATG Post-op: BAX	7d	ECU + MP RTX + IVIg ECU RTX + IVIg	9 m	ABMR resolution
Orandi 2014	5	Pre-op: PP + RTX Intra-op: 4# ATG / 1# DAC	6d	PP + IVIg ECU	1y	4# Graft loss 1# ABMR resolution
	5	Pre-op: 1# PP / 4# PP + RTX Intra-op: 4# ATG / 1# BAX	10d	PP + IVIg Splenectomy + ECU	1y	5# ABMR resolution
Tan 2019	15	Pre-op: 1# PP + RTX Intra-op: 10# ATG / 5# ALE	10d	11# PP + ECU 1# PP + ECU + Splenectomy 3# ECU	1y	6# ABMR resolution 2# active ABMR 1# chronic ABMR 1# CMR 5# NA
Norville 2023	13	NA	8d	PP + IVIg ECU	1y	3# Graft loss 9# ABMR resolution 1# chronic ABMR
Heo 2022	7	Pre-op: 4# PP + IVIg Intra-op: 5# ATG / 2# BAX	6y	ECU	1y	7# chronic ABMR
	4	Pre-op: 1# PP + IVIg Intra-op: 1# ATG / 3# BAX	2y	4# PP + IVIg 1# ECU	1y	2# ABMR resolution 2# chronic ABMR
Siddiqui 2022	2	MP + TAC + MMF	11 m / 2y	MP + PP + IVIg + RTX ECU	NA	2# ABMR resolution
Koslik 2022	61	NA	NA	PP + IA + IVIg ECU	3y	NA
Sendogan 2019	1	NA	NA	PP + IVIg ECU	5y	NA
Yamamoto 2017	1	Pre-op: PP + RTX Intra-op: BAX	2d	MP + IVIg + RTX ECU	3y	ABMR resolution
Khan 2015	1	NA	8d	MP + ATG + PEX + IVIg ECU	4 m	ABMR resolution
Kulkarni 2017	10	NA	NA	ECU	6 m	GF stable
Tran 2016	1	Intra-op: BAX	6 m	MP + PP + IVIg + RTX + ECU	1y	ABMR resolution
Orandi 2016	1	Pre-op: PP + IVIg Intra-op: ATG Post-op: PP + IVIg	5d	PP + IVIg ECU Splenic irradiation	1y	ABMR resolution
	1	Pre-op: PP + IVIg Intra-op: ATG + RTX Post-op: PP + IVIg	7d	PP + IVIg + ECU Splenic irradiation	9 m	ABMR resolution
Yelken 2015	1	Pre-op: PP + IVIg Intra-op: BAX	2d	MP + PP + IVIg + RTX + ECU	2 m	Graft loss
	1	Pre-op: PP + IVIg Intra-op: ATG	3d	MP + PP + IVIg + RTX + ECU	2y	ABMR resolution
	1	Intra-op: BAX	2d	MP + PP + IVIg + RTX + ATG + ECU	2y	ABMR resolution
	1	Intra-op: BAX	8 m	MP + PP + IVIg + RTX + ATG + ECU	2y	ABMR resolution
	1	Intra-op: BAX	9 m	MP + PP + IVIg + RTX + ATG + ECU	3 m	Graft loss
	1	Intra-op: BAX	18 m	MP + PP + IVIg + RTX + ECU	2y	ABMR resolution
	1	Pre-op: PP + IVIg Intra-op: ATG	7 m	MP + PP + IVIg + RTX + ECU	14d	Graft loss
	1	Pre-op: PP + IVIg Intra-op: ATG	1y	MP + PP + IVIg + RTX + ATG + ECU	3 m	Graft loss
Vo 2015	2	Pre-op: IVIg + RTX + PEX Intra-op: NA Post-op: IVIg	3y	IVIg + RTX ECU	5y	2# ABMR resolution
Burbach 2014	2	Pre-op: PEX + IVIg Intra-op: ATG Post-op: PEX + IVIG	1y	ECU MP + PEX + IVIg + RTX	2y	Graft Loss
		Pre-op: BOR + IVIg + RTX Intra-op: ATG Post-op: IA	1 m	IA + IVIg ECU	2y	Graft Loss
Ghirardo 2013	1	Pre-op: PP + IVIg + RTX Intra-op: ATG	1 m	MP PP + IVIg ECU	2y	ABMR resolution
Kocak 2013	1	Pre-op: PP + IVIg Intra-op: BAX	2d	MP + PP + IVIg + ALE + RTX + ECU	2 m	ABMR resolution

(*continued on next page*)

Table 1 (*continued*)

Reference (Year)	KT (n)	Immunosuppression	ABMR timing	ABMR treatment	FU	Outcomes
	1	Pre-op: PP + IVIg Intra-op: BAX	3d	MP + PP + ATG + IVIg + RTX + ECU	6 m	Graft loss
González-Roncero 2012	2	Intra-op: ATG	7d / 8d	MP + PP + IVIg + ECU + RTX	1y	2# ABMR resolution
Noonel 2012	1	Pre-op: IVIg Intra-op: ATG	7d	IVIg ECU RTX	6 m	Graft loss
NasrAllah 2022	1	NA	NA	OBI	NA	Graft loss

Abbreviations: KT, kidney transplant; n, number; ABMR, antibody-mediated rejection; FU, follow-up; PP, plasmapheresis; IVIg, intravenous polyclonal immunoglobulin; DAC, daclizumab; d, day; ECU, eculizumab; RTX, rituximab; m, month; BAX, basiliximab; ATG, anti-thymocyte globulin; MP, methylprednisolone; y, year; ALE, alemtuzumab; CMR, cell-mediated rejection; NA, not available; IA, immunoadsorption; GF, graft function; OBI, obinutuzumab.

reducing the risk of CMV, Ebstein Barr Virus, or Polyomavirus-BK infection [56]. Accordingly, we are now running an internal, exploratory study evaluating a fast desensitization-induction regimen containing eculizumab, IVIg, and obinutuzumab in highly sensitized deceased-donor KT recipients, with promising short-term outcomes [57].

Undoubtedly, the type-I anti-CD20 moAb rituximab has changed the management of some relapsing renal diseases [58] and has contributed to the development of modern desensitization [59] and anti-rejection protocols [60]. Nevertheless, the preferred use of obinutuzumab over rituximab for the treatment of ABMR may recognize several reasons. Obinutuzumab is a humanized, glycoengineered, IgG1 moAb targeting the type-II epitope of CD20, regularly expressed on pre-B cells, mature B-cells, and plasma blasts. Although rituximab and obinutuzumab bind bivalently to CD20, they form distinct complexes. Rituximab ends up stabilizing CD20 in cellular membranes, determining stronger complement binding. Obinutuzumab induces homotypic aggregation and does not stabilize CD20, thus showing lower complement-binding capacity but enhanced cell-to-cell interaction. The latter ability is further promoted by the glycoengineered Fc segment which has higher affinity to FcγRIII, displayed by macrophages and NK cells. Unlike rituximab, obinutuzumab B-cell depletion is not related to CDC, rather operating through antibody-dependent cell-mediated cytotoxicity and direct cell death (caspase independent) [61,62]. Such remarkable difference between obinutuzumab and rituximab, or other depleting agents like the anti-CD52 moAb alemtuzumab [63,64] and the anti-CD38 moAb daratumumab [65,66], makes obinutuzumab the perfect candidate for prophylactic and therapeutic regimens including complement inhibitors [67]. To date, experience with obinutuzumab for the treatment of ABMR is limited to a single unsuccessful case, briefly reported without relevant clinical or laboratory information [19]. However, data retrieved from desensitization studies suggest more powerful peripheral B-cell depletion than rituximab, with a similar safety profile [18,19,68]. Also, there is evidence that obinutuzumab has greater efficacy than rituximab in depleting lymph nodes and splenic B cells [61,62,69]. Interestingly, a wider effect on several B-cell subsets has been reported, rising hope for future immunosuppressive strategies directed toward centrally located and memory B-cells [70].

The association between detectable DSA and inferior transplant outcomes has been increasingly recognized; especially after early ABMR episodes [39,52,53]. The opportunity of reducing the burden of preformed DSA while preventing the development of de novo DSA in the long term (as observed in our experience), represents another potential benefit of obinutuzumab [39]. The impact of mild residual allograft microvascular inflammation in the absence of circulating C1q-fixing DSA on transplant survival remains debated [71]. Furthermore, as clearly stated in the Banff 2019 Kidney Meeting Report, we cannot draw conclusions regarding the significance of biopsies showing moderate microvascular inflammation (g + ptc >2) without DSA. Probably, the prognostic implications of these changes may substantially differ in case of early vs late DSA detection or between recipients with circulating preformed DSA or de novo DSA [30]. Reassuringly, after eculizumab and obinutuzumab administration, allograft biopsies did not show signs of moderate-to-severe glomerulitis (g > 1) or peritubular capillaritis (ptc >1), with undetectable C1q-fixing DSA or de novo DSA. The observation that our patients remained negative for intra-allograft C4d and MAC deposition, maintaining stable renal function and normal liquid biopsy up to three years of follow-up, is certainly encouraging, but larger studies with matched control groups and extended histology data are warranted to validate our preliminary results and properly assess the effects of obinutuzumab on chronic active ABMR and long-term transplant survival. A wider analysis focused on treatment safety and treatment-related cost is also advisable. In this regard, considering the rarity of the condition, the heterogeneity of the population, and the differences in clinical practice among transplant centers worldwide, the implementation of national and international registries with detailed descriptions of anti-rejection strategies and standardized records of patient- and transplant-related outcomes would be extremely helpful.

Finally, when assessing the possible role of obinutuzumab in current transplant practice, the progressive loss of efficacy observed with repeated rituximab administration (mostly due to the development of anti-rituximab antibodies) should be considered [69,72]. In fact, as the number of patients with relapsing diseases and failed transplants increase, the proportion of KT candidates with acquired resistance to rituximab requiring desensitization, prophylaxis, or treatment with alternative anti-CD20 moAb will rise significantly. Both patients described here had idiopathic FSGS, a rare condition causing nephrotic syndrome with high recurrence rates after KT. Even though the exact pathogenesis remains unclear, it is accepted that a not yet identified circulating factor may cause podocyte injury and progression to glomerulosclerosis [54]. For many years, plasmapheresis has represented the mainstay of treatment. More recently, improved outcomes have been achieved with type-1 anti-CD20 moAb. The rationale behind the use of B-cell depleting agents for the prevention or treatment of relapsing FSGS is that they might inhibit the production of the circulating factor [54]. To date, rituximab represents the preferred option, with ofatumumab administered in different doses and combinations in case of rituximab-resistant disease. Experience with obinutuzumab is basically limited to a single study evaluating the combination of obinutuzumab and daratumumab in patients with multidrug dependent nephrotic syndrome [73]. In theory, transplant recipients with idiopathic FSGS could particularly benefit from prophylactic obinutuzumab compared to transplant candidates with other PRD, as the type-2 anti-CD20 moAb might reduce relapse rates or improve response to treatment in case of recurrence. However, in line with current international guidelines [40], we believe that the choice of ABMR protocol should not be guided by the PRD of the recipient unless drug-related adverse events are anticipated, or signs of concomitant relapsing disease are detected. Indeed, the fact that our patients had idiopathic FSGS does not compromise the generalizability of the anti-rejection scheme herein proposed as eculizumab and obinutuzumab can be virtually administered to all KT recipients experiencing ABMR regardless of their PRD. Importantly, in our series,

the occurrence of post-transplant relapsing FSGS (at the time of anti-rejection treatment and during the entire follow-up) was conclusively ruled out by repeated clinical evaluations, allograft biopsies, and laboratory tests. Therefore, it remains extremely difficult to speculate about potential relationships or interactions between idiopathic FSGS, post-transplant relapse, ABMR, and treatment-related outcome.

The need for more effective anti-rejection protocols cannot be emphasized enough. As a matter of fact, 3-year allograft survival rates lower than 50% have been reported in KT recipients with ABMR treated with the current standard of care [55]. Because most studies describe different immunosuppressive schemes and do not provide quantitative data on B-cell count, DSA levels, patients' safety, or treatment-associated cost, comparing statistics would remain of limited use [40]. On the contrary, we believe that reviewing the results from our historical cohorts of patients could provide valuable background information. In five years, 20 subjects with characteristics like those of the patients herein described, have received a deceased-donor KT at out institution (**Supplementary Table 2**). Peri-transplant conditioning included IV steroid, rATG (5 mg/kg/ total-dose), PEX (three-to-five sessions), and IVIg (2 g/kg total-dose) in all cases. Eight patients were also given rituximab (375 mg/m^2) as a desensitization or induction agent. Overall, five episodes of early ABMR due to preformed DSA were recorded (incidence, 25%). First-line anti-rejection treatment consisted of IV steroid, PEX (three-to-five sessions), and IVIg (2 g/kg total-dose). Only one patient showed complete response, with reversal of active ABMR on allograft histology and preserved renal function up to one year of follow-up. For the remainder, we used repeated PEX, IVIg, and rituximab ($n = 2$) as a rescue therapy. Eventually, 3/4 (75%) recipients experienced allograft loss within six months of transplant; the other one developed chronic active ABMR with progressive deterioration of function. After rituximab administration, no serious IRR, life-threatening infectious complications, or malignancies were observed. A transient reduction in circulating anti-HLA antibody levels was achieved in all patients. However, most DSA (especially Class-II) remained >1000 MFI. Although the results might have been influenced by the withdrawal of MMF, signs of B-cell recovery (peripheral CD20+ count >1%) could be detected as early as six months after infusion. Our most recent experience with a small group ($n = 7$) of KT recipients diagnosed late ABMR and treated with apheresis (five sessions), IVIg (2–4 g/kg total-dose), and rituximab (375 mg/m^2) was also disappointing (**Supplementary Table 3**). Indications for biopsy were rise in SCr, abnormal 24-h proteinuria, and/or detection of circulating DSA >1000 MFI. Five patients presented with isolated Class-II DSA whereas two recipients exhibited both Class-I and Class-II DSA. After treatment, we observed a transient improvement of estimated glomerular filtration rate (eGFR) in five recipients. However, extending the follow-up over the first year, most patients (6/7, 86%) showed a progressive deterioration of renal function. A significant reduction (≥50%) in DSA levels was achieved in 3 patients. Remarkably, median MFI remained persistently >3000 in all cases. No severe IRR, infections, or hematologic complications were recorded. One recipient experienced an allergic reaction during plasmapheresis.

Considering the limited information available, as well as the safety profile and cost of eculizumab and obinutuzumab, we believe that the use of this novel anti-rejection scheme should be currently guided by the specific needs and clinical conditions of the patients. In particular, the risk of infusion-related reactions, drug-induced side effects, infections, and malignancy (overall acceptable but not neglectable) should be weighed against the risk of transplant loss (and death due to return to dialysis) associated with ABMR. In this regard, waiting for more robust data, it may be reasonable to enroll patients with active ABMR who have failed to respond to the standard of care or perhaps patients with early ABMR and limited changes of further transplants. The prophylactic administration of eculizumab and obinutuzumab in highly sensitized kidney transplant recipients with exceedingly high risk of ABMR (elevated preformed DSA) might represent another feasible option and it is currently under evaluation at our institution. The results of ongoing clinical trials in patients with idiopathic FSGS or other nephrotic syndromes may clarify whether obinutuzumab could be used prophylactically to prevent disease recurrence after transplant.

6. Conclusion

Our experience suggests that obinutuzumab represents a feasible option when aiming to achieve prompt and long-lasting peripheral B-cell depletion and DSA production blockage in highly sensitized KT recipients with early ABMR. Furthermore, it confirms that eculizumab can stop (at least temporarily) complement-dependent DSA-mediated allograft injury. When using eculizumab as a part of multimodality prophylactic or treatment strategies, serial monitoring of complement activity is recommended, as drug efficacy can be substantially impaired by apheresis and plasma substitution. Relevantly, the data herein reported further demonstrate that obinutuzumab peripheral B-cell depleting properties are not affected by concomitant inhibition of the terminal complement cascade, representing the first clinical evidence supporting the preferential use of obinutuzumab over type-1 anti-CD20 agents in patients with ABMR previously exposed to complement inhibitors. Despite the novelty and the encouraging results, we recognize the limitations of the present work; in particular, the lack of randomization, the use of small heterogeneous historical control groups, the complexity of the first-line treatment adopted, and the absence of long-term protocol allograft biopsies for the evaluation of possible effects on the development of chronic active ABMR. Further studies with better design and larger populations are certainly needed to confirm the clinical efficacy and safety of eculizumab and obinutuzumab for the treatment of ABMR. Nevertheless, current trends seem to favor obinutuzumab over rituximab in upcoming clinical research projects.

Ethics statement

The subjects involved have formally consented for enlistment in the kidney transplant waiting list, kidney transplantation, transplant-related treatments (including label and off-label use), and follow-up investigations. The anti-rejection protocol and the retrospective collection and use of anonymized clinical data for publication purpose have been approved by the local authorities and by the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Ethical Committee (Protocol ID: OBIKIT2023 - OSMAMI-16/03/2023–0011790-U). The study was conducted according to the World Health Organization Declaration of Helsinki and applicable regulatory requirements. Participants were informed of the potential risks and benefits deriving from the off-label use of obinutuzumab and eculizumab and signed a specific consent form.

Declaration of competing interest

The authors of this manuscript have no conflicts of interest to disclose.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request (article evaluation and/or formal research purpose).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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