The FcγRIIIA-158 VV genotype increased the risk of post-transplant lymphoproliferative disorder in T-cell-depleted kidney transplant recipients – a retrospective study

P. Gatault, L. Lajoie, J. Stojanova, J.-M. Halimi, S. Caillard, S. Moyrand, D. Martinez, M. Ladrière, E. Morelon, P. Merville, et al.

DR. PHILIPPE GATAULT (Orcid ID : 0000-0003-1664-2879)

PROF. NASSIM KAMAR (Orcid ID : 0000-0003-1930-8964)

Article type : Original Article

The FcγRIIIA-158 VV genotype increased the risk of post-transplant lymphoproliferative disorder in T-cell depleted kidney transplant recipients.

Authors:

Philippe Gatault[1,2], Laurie Lajoie[3], Jana Stojanova[4], Jean-Michel Halimi[1], Sophie Caillard[5], Stéphanie Moyrand[6], David Martinez[6], Marc Ladrière[7], Emmanuel Morelon[8], Pierre Merville[9,10], Marie Essig[4,11], Cécile Vigneau[12], Nassim Kamar[13], Nicolas Bouvier[14], Pierre-François Westeel[15], Christophe Mariat[16], Marc Hazzan[17,18], Antoine Thierry[19], Isabelle Etienne[20], Matthias Büchler[2], Pierre Marquet[4], Valérie Gouilleux-Gruart[3,6], Gilles Thibault[3,6].

Affiliations:

[1]: EA4245, T2I, University of Tours, 10 Boulevard Tonnelle, 37032 Tours France. [2]: Department of Nephrology and Clinical Immunology, University Hospital, Tours, France. [3]: EA7501 « Groupe Innovation et Ciblage Cellulaire » team « Fc Receptors, Antibodies and Microenvironment », University of Tours, 10 Boulevard Tonnelle, 37032 Tours France. [4]: Pharmacology and transplantation, Inserm, Univ Limoges, CHU Limoges, FHU SUPORT, Limoges, France. [5]: Department of Nephrology, University Hospital of Strasbourg, Strasbourg, France.[6]: Immunology Laboratory, University Hospital, Tours, France. [7]: Department of Nephrology, University Hospital, Vandœuvre-lès-Nancy, France. [8]: Department of Transplantation, Nephrology and Clinical Immunology, Hopital Edouard Herriot, Hospices Civils de Lyon, Lyon, Université Lyon 1, Inserm U 1111, France. [9]: Service de Néphrologie-Transplantation-Dialyse-Aphérèses CHU

Bordeaux, Bordeaux, France. [10]: CNRS-UMR 5164 Immuno ConcEpT Université de Bordeaux, 33076 Bordeaux, France. [11]: CHU Limoges, Service de néphrologie, dialyse-transplantations, Limoges, France. [12]: Department of Nephrology, Pontchaillou University Hospital, Rennes, France. [13]: Departments of Nephrology and Organ Transplantation, CHU Rangueil, INSERM U1043, IFR–BMT, Université Paul Sabatier, Toulouse, France. [14]: Department of Nephrology and Renal Transplantation,University Hospital, Caen, France. [15]: Department of Dialysis and Transplantation, University Hospital, Amiens; and INSERM ERI-12, University of Picardie – Jules Verne, Amiens, France; [16]: Service de Néphrologie, Dialyse, Transplantation Rénale, Hôpital Nord, CHU de Saint-Etienne, GIMAP, EA 3065, Université Jean Monnet, Saint-Etienne, Comue Université de Lyon, Lyon, France. [17]: Service de Néphrologie - CHRU de Lille - F-59000 Lille, France. [18]: Université de Lille - UMR 995, F-59000 Lille, France. [19]: Department of Nephrology, Jean Bernard Hospital,University Hospital, Poitiers, France. [20]: Department of Nephrology, University Hospital, Rouen.

Authors' Contributions:

Philippe Gatault: literature search, figures, study design, data collection, data analysis, data interpretation, writing

Laurie Lajoie: figures, data collection, data analysis, data interpretation, writing

Jana Stojanova: data collection

Jean-Michel Halimi: data analysis, data interpretation, writing

Sophie Caillard: data interpretation, writing

Stéphanie Moyrand: data collection, data analysis

David Martinez: data collection

Marc Ladrière: data collection, writing

Emmanuel Morelon: data collection, writing

Pierre Merville: data collection, writing

Marie Essig: data collection, writing

Cécile Vigneau: data collection, writing

Nassim Kamar: data collection, writing

Nicolas Bouvier: data collection, writing

Pierre-François Westeel: data collection, writing

Christophe Mariat: data collection, writing

Marc Hazzan: data collection, writing

Antoine Thierry: data collection, writing

Isabelle Etienne: data collection, writing

Matthias Büchler: data collection, writing

Pierre Marquet: study design, data collection, data analysis, writing

Valérie Gouilleux-Gruart: study design, data analysis, writing

Gilles Thibault: study design, data collection, data analysis, data interpretation, writing

Funding*:* "Investissement d'avenir programs, Grant Agreement LabExMAbImprove: ANR-10-LABX-53"

Running title: Genetic risk of PTLD after T-cell depletion

Corresponding author:
Dr Philippe Gatault, MD, PhD
Department of Nephrology and Clinical Immunology
10, Bd Tonnelle. 37032 Tours FRANCE
Phone: +33 (0) 2 47 36 61 22
Fax: +33 (0) 2 34 37 89 01
E-mail: philippe.gatault@univ-tours.fr

Abbreviations.

ADCC, antibody-dependent cell-mediated cytotoxicity

ALG, anti-lymphocyte globulin

EBV, Epstein–Barr virus

IgG, immunoglobulin G

FcγRIIIA, Fc gamma receptor IIIA (

FITC, fluorescein isocyanate

mAb, monoclonal antibody

NK cell: natural killer cell

PTLD, post-transplantation lymphoproliferative disorder

pTLDAb, polyclonal T lymphocyte-depleting antibodies

rATG: rabbit antithymocyte globulin

TLDAb, T lymphocyte-depleting antibodies

Abstract

Background. Post-transplantation lymphoproliferative disorder (PTLD) is a severe complication in organ transplant recipients. The use of T lymphocyte-depleting antibodies (TLDAb), especially rabbit TLDAb contributes to PTLD and the V158F polymorphism of Fc gamma receptor IIIA (FcγRIIIA) also named CD16A could affect the concentration–effect relationship of TLDAb. We therefore investigated the association of this polymorphism with PTLD in kidney transplant recipients.

Methods. We characterized the V158F polymorphism in two case–control cohorts (discovery, n=196; validation, n=222). Then, we evaluated the binding of rabbit IgG to human FcγRIIIA-158V and -158F.

Results. The V158F polymorphism was not linked to PTLD in the overall cohorts, but risk of PTLD was increased in VV homozygous recipients receiving TLDAb compared to F carriers in both cohorts, especially in recipients receiving TLDAb without muromonab (discovery: HR=2.22 [1.03-4.76], p=0.043, validation: HR=1.75 [1.01-3.13], p=0.049). *In vitro,* we found that the binding of rabbit IgG to human NK cell FcγRIIIA was increased when cells expressed the 158-V versus the 158-F allotype.

Conclusions. While the 158-V allotype of human FcγRIIIA binds rabbit immunoglobulin-G with higher affinity, the risk of PTLD was increased in homozygous VV kidney transplant recipients receiving polyclonal TLDAb.

Keywords: Solid organ transplantation, Post-transplant lymphoproliferative disorder, T lymphocyte-depleting antibodies, Fc gamma receptor IIIA, Gene polymorphism

Introduction

Post-transplant lymphoproliferative disorder (PTLD) is the second most frequent malignancy in organ transplant recipients. The incidence of PTLD is increased in the first post-transplant year, but the risk remains beyond the first year (1-4). Indeed the 10-year cumulative incidence is close to 2%, about 10 to 20 times higher than in the general population (1, 4, 5). The mortality and the risk of graft loss remain high after PTLD (5-7). To prevent PTLD, we must identify individual pre-transplantation PTLD risk factors. By now, the best described risk factors of PTLD are the seronegative EBV status at the time of transplantation and the immunosuppression, that can affect the antiviral T cell response (8, 9). However, their clinical significance remains relatively limited because a large majority of adults recipients is infected by EBV before the transplantation and the contribution of each immunosuppressive drug is difficult to determine.

Induction therapy using T lymphocyte-depleting antibodies (TLDAb) was found associated with increased incidence of PTLD (1, 4, 9, 10). The mechanisms underlying this risk remain unknown, although an impaired antiviral response after T-cell depletion is usually hypothesized from the following findings: 1) the risk of PTLD was found reduced after treatment with polyclonal TLDAb (pTLDAb) like rabbit antithymocyte globulin (rATG) or horse anti-lymphocyte globulin (ALG) versus mouse muromonab-CD3 antibodies, which induces greater T-cell depletion (11); and 2) a dose reduction of TLDAb in modern induction regimens has been associated with reduced PTLD incidence (4, 9). pTLDAb induces lymphocyte depletion in the peripheral blood primarily by complement-dependent cell lysis, activation-induced cell death and antibody-dependent cell-mediated cytotoxicity (ADCC) (12), involving the Fc gamma receptor IIIA (FcγRIIIA). In human, this low-affinity receptor for the Fc fragment of immunoglobulin G (IgG) links IgG-sensitized target cells to FcγRIIIA-bearing cytotoxic cells, such as natural killer (NK) cells and monocytes/macrophages leading to target cell lysis.

The *FCGR3A* gene, encoding the FcγRIIIA, features a functional allelic polymorphism (rs 396991) generating allotypes with a phenylalanine (F) or a valine (V) at amino acid position 176 (13, 14). The FcγRIIIA-158V allotype has a higher affinity for human IgG (especially IgG1 and IgG3), associated with enhanced clinical response to several therapeutic human IgG1 monoclonal antibodies (15-18). Interestingly, Ternant *et al.* recently reported that kidney transplant recipients with homozygous VV genotype exhibited higher T cell depletion upon treatment with horse and rabbit pTLDAb (19, 20). While it has been reported that the V158F polymorphism is not

associated with altered risk of developing PTLD in organ transplant recipients without T-cell depletion (21), this has not been evaluated in recipients receiving pTLDAb, whose proportion is increasing (22). The aim of the present study was therefore to assess the effect of the V158F polymorphism on the risk of PTLD in kidney transplant recipients, with a focus on those who have received pTLDAb.

Material and methods

Patients

Discovery cohort

Among kidney recipients who underwent renal transplantation in our unit between 1985 and 2015 (n=2107), we identified 52 patients with PTLD and DNA samples were available for 49 of them. Ethics committee approval was granted for the collection of DNA samples and the study was approved by the Comité de Protection des Personnes Tours-Région Centre Ouest 1 (DC 2013-1780). Then, we selected 3 recipients without PTLD for each recipient with PTLD with the nearest date of transplantation because dosing strategies of ATG and ALG have greatly changed over time. Date of death, graft loss or last visit was considered as the censoring date. Among the 196 matched recipients 147 had received pTLDAb.

Validation cohort (Figure 1)

We obtained DNA samples extracted from peripheral blood or tumoral tissue in 278 matched recipients (1:1) from a French multicentric case–control study limited to recipients receiving transplants since 1990. Cases and controls were matched one-to-one on transplant centre, sex, age at transplantation (+/- 5 years), year of transplantation (+/- 1 year), graft order (1st, 2nd or 3rd transplant), and pre-transplant EBV status. To approximate time-to-event analysis, controls were selected to have the same time length of immunosuppression as the transplant-to-PTLD period in cases. We excluded 16 matched recipients already included in our discovery cohort and genotyping was not feasible in 19 recipients (38 matched recipients were therefore excluded). Finally, we excluded 2 matched recipients because PTLD was recently diagnosed in a control recipient. Among the 222 matched recipients 129 had received pTLDAb.

FCGR3A genotyping

Single-step multiplex allele-specific PCR assays were performed as previously described (23) with minor modification. The 25-µL reaction mixture contained 10 ng genomic DNA, 400 nM forward primer (5'-TCCAAAAGCCACACTCAAAGTC-3'), 400 nM reverse V allele primer (5'-AGACACATTTTTACTCCCATC-3') and 200 nM reverse F allele primer (5'-

GCGGGCAGGGCGGCGGGGGCGGGGCCGGTGATGTTCACAGTCTCTGATCACACATTT TTACTCCCATA-3'), 400 μM of each dNTP, 2 mM $MgCl_2$ and 0.5 U Taq DNA polymerase in its buffer (Promega). PCR conditions were 3.5 min at 95°C followed by 35 cycles, each at 95°C for 20 sec, 56°C for 20 sec, 72°C for 30 sec. Next, PCR products (137- and 81-bp fragments for F and V alleles) were resolved on 8% acrylamide gel (Invitrogen) and visualized after ethidium bromide staining.

Effect of the V158F polymorphism on rabbit polyclonal IgG binding to human FcγRIIIA

CD16-transduced NK-92 cell lines (FcγRIIIA-158V or -158F) used to evaluate binding to FcγRIIIA were kindly provided by Dr. B. Clémenceau (INSERM CR 1232 CRCINA, Nantes, France). Cells were cultured in RPMI-1640 medium (Sigma) supplemented with 10% fetal bovine serum (PAA Laboratories), 100 UI/ml interleukin 2 (Proleukin, Chiron Corp.), 2 mM L-glutamine (Sigma), penicillin (100 UI/ml) and streptomycin (0.1 μg/ml) (Sigma) at 37 °C under a humidified 5% CO_2 atmosphere. FITC-conjugated anti-CD16 monoclonal antibody (mAb) clone 3G8 and rabbit polyclonal IgG (pIgG) were from Beckman Coulter and Sigma, respectively. Tegeline® (human pIgG) was kindly provided by LFB.

The mAb 3G8 (mouse IgG1) recognizes an epitope within the binding site of human IgG. Therefore, it inhibits binding to FcγRIIIA and was previously used to evaluate the binding of rituximab to FcγRIIIA by flow cytometry (24). We evaluated the ability of rabbit or human pIgG to inhibit the binding of FITC–conjugated 3G8 to the FcγRIIIA-expressing NK-92 cell line. Briefly, CD16-transduced NK-92 cells (FcγRIIIA-158V or -158F) (2×10^5 in 100 μL) were incubated with FITC–conjugated 3G8 (dilution 1:100) for 30 min at 4°C. Cells were then incubated for 30 min at 4°C with 0.01 to 10 mg/mL of rabbit or human pIgG and analyzed by flow cytometry. All flow cytometry analyses were performed with 10,000 events with a Gallios flow cytometer and Kaluza 1·3 software (Beckman Coulter). The results are expressed as percentage inhibition of 3G8 binding: (% of 3G8-positive NK-92 FcγRIIIA-158V or -158F cells in the absence of pIgG − % of 3G8-positive NK-92 FcγRIIIA-158V or -158F cells in the presence of pIgG) × 100 ÷ % of 3G8-positive NK-92 FcγRIIIA-158V or -158F cells in the absence of pIgG.

Statistical analysis

Data are expressed as percentage or mean ± SD for normally distributed variables and median (range) for non-normally distributed variables. Qualitative data were compared by chi-square test. Quantitative data were compared by Mann–Whitney U test. A cox regression was performed to

identify the risk factors of PTLD in the discovery cohort. We successively assessed all recipients (n=196), recipients receiving pTLDAb with (n=147) or without (n=141) muromonab-CD3 antibodies, which is no longer used but was known to represent a strong risk factor of PTLD. Second, we adjusted the association between V158F polymorphism and PTLD for recipient age, type of pTLDAb (rATG or ALG) and recipient EBV serology. We carried out a similar analysis with three successive groups in the validation cohort with an adjustment for recipient age and recipient EBV serology (but not duration and type of pTLDAb because this information was not available in the validation cohort). Results are expressed as hazard ratios (HRs) and 95% confidence intervals (95% CIs). We additionally generated Kaplan-Meier curves to compare actuarial PTLD-free survival (censored at 20 years) in VV, VF and FF recipients from both cohorts receiving pTLAD without muromonab-CD3, in order to assess a potential dose-effect of V15F polymorphism on risk of PTLD. Curves were compared using Log-rank test. To evaluate the effect of the V158F polymorphism on rabbit pIgG binding to FcγRIIIA, simple linear regression was calculated by the method of least squares for each inhibition to compare the half-maximal inhibitory concentration (EC_{50}). Statistical analyses involved use of SAS v9.1 (SAS Inst., Cary, NC). $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of the discovery and validation cohorts

The characteristics of cases (PTLD) and controls (no PTLD) in both cohorts are presented in Table 1. Cases and controls did not differ in age and had a similar proportion younger than 18 years in the discovery cohort (cases: 3/49, controls: 8/147, p = 1) and the validation cohort (cases: 1/111, controls:0/111, p = 1). Otherwise, cases and controls did not differ in demographic data, incidence of acute rejection and maintenance immunosuppressive regimen, except for a slightly higher proportion of cases than controls receiving azathioprine in the validation cohort. Importantly, the proportion of patients seronegative for EBV before transplantation was not different in VV and F-carriers in discovery cohort (7.2% vs. 10.4%, p=1) and validation cohort (13.5% vs. 12.4%, p=0.857).

In the discovery cohort, 147 (75%) recipients received pTLDAb (rATG and/or ALG). Except for 2 recipients with steroid-resistant rejection, pTLDAb were used as induction therapy. Most recipients received rATG (n=137, 93.2%), combined with (n=42: cases=11, controls=31) or without ALG (n=95, cases=23, controls=72). Ten recipients received ALG without rATG (cases=2, controls=8). Overall, cases and controls did not differ in use of pTLDAb. In the

validation cohort, 129 (58%) recipients received pTLDAb (rATG: 114), with a higher proportion in cases than in controls.

Increased risk of PTLD in homozygous VV kidney transplant recipients receiving pTLDAb

Discovery cohort

Among the 196 recipients, 67, 34 and 95 were homozygous for FcγRIIIA-158F (FF), homozygous for FcγRIIIA-158V (VV) and heterozygous (VF), respectively. Thus, the frequency of the V allele was 41.3%, respecting the Hardy-Weinberg equilibrium (p = 0.997). The median delay between transplantation and PTLD diagnosis was 4.9 years (range 0.1 to 20.5). On univariate analysis in the overall cohort, the V158F polymorphism was not significantly linked with PTLD and muromonab-CD3 antibody treatment was the sole risk factor of PTLD (Table 2). In 147 recipients receiving pTLDAb, we found that VV homozygous recipients had a higher risk of PTLD (HR=2.08 [95% CI 1.01-4.35], p=0.046), whereas muromonab-CD3 antibody treatment remained strongly associated with PTLD (HR=4.61 [1.62-13.06], p=0·004). Finally, the VV genotype was still associated with PTLD in the 141 recipients receiving pTLDAb without muromonab-CD3 antibodies as shown in Table 2 (HR=2.22 [1.03-4.76], p=0.043). This greater risk remained significant after adjustments on recipient age, type of pTLDAb and recipient EBV serology (HR=3.44 [1.19-11.11], p=0.023).

Validation cohort

Among the 222 recipients, 72, 37 and 113 were FF, VV and VF, respectively. Thus, the frequency of the V allele was 42.1%, respecting Hardy-Weinberg equilibrium (p = 0.898). The time between transplantation and PTLD occurrence (6.49 [0.11-25.28] years) did not differ from that in the discovery cohort (p=0.186). We confirmed on univariate analysis that the V158F polymorphism was not significantly linked with PTLD in the overall cohort (HR= 1.37 [0.78-2.56], p=0.268), but was significantly associated with PTLD in 129 recipients receiving pTLDAb, especially in 127 recipients who did not receive muromonab-CD3 antibodies (Table 3, HR=1.75 [1.01-3.13], p=0.049). This association remained significant after adjustments for recipient age and recipient EBV serology (HR=1.92 [1.02-3.71], p=0.044).

We additionally raised the question of V158F polymorphism dose-effect in all patients receiving pTLDAb but not muromonab-CD3 antibodies (discovery and validation cohorts where pooled for this analysis, while we failed to show a difference between VV , VF and FF in the discovery cohort). We assessed 48 VV (25 from discovery cohort/23 from validation cohort), 134 VF (67/67) and 86 FF (49/37) (distribution of patient in cases and controls is provided in

supplementary data, Table S1). As shown in Figure 2, we observed that risk of PTLD was different in three groups (p=0.026), with the highest risk observed in VV recipients (VV vs. VF: p=0.029; VV vs. FF: p=0.011; vs F-carrier: p=0.010). Risk of PTLD in FF and VF recipients was not different (p=0.460).

Characteristics of PTLD in discovery and validation cohorts

PTLD characteristics were quite similar in the two cohorts (Table 4A). The only difference concerned the localization of the tumor with a lower proportion of primary central nervous in the validation cohort PTLD, balanced by a higher percentage of PTLD within the kidney graft. We then assessed the PTLD characteristics by V158F polymorphism (Table 4B). We did not observe any differences in two cohorts with regard to the localization, the tumor EBV status and the cell type involved, that was almost exclusively B cells irrespective of the genotype. Additionally, the proportion of early-onset PTLD did not differ between VV homozygous and F-carrier recipients. Finally, PTLD characteristics did not differ by V158F polymorphism in recipients treated with pTLDAb (data not shown).

Effect of the V158F polymorphism on binding of rabbit pIgG to human FcγRIIIA.

We wondered whether the association between the V158F polymorphism and the susceptibility to rATG in kidney transplant recipients may be related to a difference in rATG binding to human FcγRIIIA. To address this question, we used our previously described flow cytometry assay (23) to compare the *in vitro* ability of rabbit pIgG to inhibit the binding of FITC-conjugated 3G8 mAb on the human NK-92 cell line expressing the V or F allotype of FcγRIIIA. rATG could not be used in this assay because it contains antibodies for human NK-cell membrane antigens. As expected and previously reported with rituximab and purified NK cells (23), we found a higher ability of human pIgG to inhibit the binding of 3G8 mAb to FcγRIIIA on NK-92 cells expressing the 158-V versus the 158-F allotype: 50% inhibition of 3G8 mAb binding (EC50) was achieved on average with 0.09 and 0.29 mg/mL pIgG (n=3), respectively (Figure 3). Results obtained with rabbit pIgG were similar: 50% inhibition of 3G8 mAb binding to FcγRIIIA-158V– and -158F–expressing NK-92 cells was achieved with 0.04 and 0.2 mg/mL pIgG, respectively. Thus, these findings unambiguously demonstrated higher binding of rabbit IgG on NK-92 cells expressing the FcγRIIIA-158V compared to cells expressing the FcγRIIIA-158F allotype.

Discussion

In this study, we report for the first time that the V158F polymorphism of FcγRIIIA is an independent risk factor of PTLD in kidney transplant recipients after polyclonal T-lymphocyte depletion therapy. Risk of PTLD was increased 2-fold in homozygous VV recipients from our monocentric cohort and was confirmed in a larger and more recent multicentric cohort designed differently. (20) Accordingly, we demonstrated *in vitro* experiments that the FcγRIIIA 158-V allotype binds rabbit pIgG more efficiently than the 158-F allotype.

So far, studies that assessed the role of the V158F polymorphism on the PTLD course have focused on the outcome following the diagnosis, and shown either an improved survival in VV patients treated or not with rituximab or the absence of influence of the V158F polymorphism on the response to rituximab (21, 25). Stern et al. failed to observe an association between the V158F polymorphism and the risk of PTLD, which might be considered as conflicting with our results at first glance (21). However, we obtained similar results in our two cohorts when all patients (*i.e.* who received or not pTLDAb) were analyzed. It is also of note that only 61 recipients received pTLDAb in the previous study. We therefore consider that results of Stern study are not conflicting with ours that restrict the effect of the V158F polymorphism on PTLD to patients with lymphocyte depletion therapy. As well, heart and liver kidney transplant recipients were included in the study of Stern et al. The association between the V158F polymorphism and PTLD should be specifically assessed in such patients, who have a greater risk of PTLD than kidney transplant recipients (26). Owing to the fact that the increased risk in our of PTLD in VV recipients is restricted to patients receiving pTLDAb, overwhelmingly rATG study, our results suggest a link between T-lymphocyte depletion and the occurrence of PTLD. We definitely demonstrated *in vitro* that the membrane FcγRIIIA-158V allotype bound rabbit IgG with a higher affinity than the FcγRIIIA-158F allotype, as was also indirectly suggested in pharmacological population studies reporting that the depletion of $CD3^+$ and $CD4^+$ lymphocytes was extended with the number of V alleles (19, 20). This biological evidence of the functionality of the V158 polymorphism with rabbit IgG as well as the replication of our clinical results in a validation cohort are equally important to consider our results as reliable and compelling. Indeed, both steps missed in studies that established an association between the risk of PTLD and the presence of a polymorphism in HLA and cytokines (TNF-α, IL-28, IL-10, TGF-β) genes, that have not been confirmed thereafter (27-30). *FCGR3A* gene polymorphism has been associated with clinical responses to different cytolytic mAbs (14-16). These pharmacogenetic studies and the present one demonstrate that FcγRIIIA-expressing cells are involved in the mechanism of action of these mAbs and of

pTLDAb. In addition, an *in vitro* genotype–phenotype association has been observed: the *FCGR3A* polymorphism affects the affinity of human mAbs (23) and that of pTLDAb (present study) for FcγRIIIA and accordingly the level of ADCC mediated by NK cells *in vitro* (16, 23). Finally, kidney transplant recipients with homozygous VV genotype exhibited higher T cell depletion upon treatment with horse and rabbit pTLDAb (19, 20). Taken together these results suggest that the increased risk of PTLD observed in VV recipients may result from a deeper T-cell depletion related to enhanced pTLDAb-mediated ADCC favoring the emergence of lymphomatous cells. It can be highlighted that we did not observe a dose-effect of polymorphism, suggested by pharmacologic study that showed an increase in sensitivity of patients to r-ATG treatment with the number of V alleles (20). A similar behavior of VF and FF patients is classically observed with monoclonal therapeutic antibodies.

PTLD are very heterogeneous and classically two types are considered based on the role of oncogenic EBV. The EBV-positive PTLDs represent 50-80 % of cases. They can occur in EBV-positive recipients but are particularly frequent in EBV negative recipients (mostly pediatric recipients) infected after the transplantation, especially by a graft harvested from a EBV-positive donor. Monitoring the EBV load is only recommended in EBV-negative recipients (31), while its usefulness in EBV-positive recipients remains debated (32, 33). As compared with EBV-driven PTLD, EBV-negative PTLDs occur more often later and feature some histological and genetic characteristics, while their proportion increases these last years (33-35). However, the impact of PTLDs on graft and patient survivals did not seem to be affected by the EBV status of the tumor (34). In our study, the association between the V158F polymorphism and the risk of PTLD was not affected after adjustment for the recipient EBV serology and the proportion of EBV-negative tumor was similar in VV and F-carrier recipients. Furthermore, we did not observe any differences regarding PTLD characteristics that could have suggested a predominant effect of the SNP on EBV-driven PTLD, in particular the proportion of early-onset disease. Determining the FcγRIIIA-V158F polymorphism before the transplantation may therefore be useful to assess the risk of both EBV-driven and EBV-negative PTLDs. Additionally, our study suggest that a deep T-cell depletion fosters the trigger of EBV-negative PTLDs, that remained largely unknown (8). Finally, because our cohort consisted mainly in EBV-seropositive adult patients, we conclude that the impact of genotyping in EBV-seronegative recipients remains to elucidate.

Importantly, we consider FcγRIIIA-V158F polymorphism characterization as clinically pertinent because VV recipients represented about 15% of our recipients, in agreement with frequencies in Caucasian individuals reported in previous studies but also because the minor allele frequency does not greatly change around the world, in Asian, Hispanic or African populations (36, 37). It is known that pTLDAb are more efficient than interleukin-2 receptor antagonists to prevent rejection in recipients at high immunologic risk (31). Therefore, we do not suggest to contraindicate use of pTLDAb in all VV recipients. However, genotyping may provide an important information to clinicians for recipients at lower immunologic risk for whom induction treatment and non-depleting interleukin-2 receptor antagonists could be used alternatively in F-carriers, whereas the former should be avoided in VV patients.

Our study has several limitations. First, the number of patients in the discovery cohort was small. However, results were confirmed in the second larger independent and differently designed cohort. Second, the analyses were performed retrospectively and some data were missing regarding the histology and the EBV status of the tumor. This weakness is nevertheless observed in the vast majority of studies regarding PTLD because it remains a rare disease (especially in kidney transplantation) occurring over a long period after the transplantation. Third, transplantation occurred over a long time, during which medical practices, particularly immunosuppressive strategies like dose and duration of TLDAb, have greatly changed. Therefore, we matched cases and controls on the date of transplantation in both cohorts. In addition, we observed that the V158F polymorphism increased the risk of PTLD in recipients with pTLDAb but not muromonab-CD3 treatment, which is no longer used. Fourth, the link between PTLD and the V158F polymorphism, which additionally affected the affinity of rabbit pIgG to FcγRIIIA, supports that the substantial post-transplant T-cell depletion may participate in subsequent development of PTLD. Nevertheless, a retrospective case-control can never definitively establish a causal relationship. Unfortunately, the evaluation of T cell depletion was not possible because CD3+, CD4+, and CD8+T lymphocytes counts before and after transplantation were missing in a large proportion of patients.

In summary, we report an increased risk of post-transplantation lymphoproliferative disorder in VV homozygous *FCGR3A* kidney transplant recipients receiving pTLDAb, especially thymoglobulin, in two independent cohorts. These results may lead to a better evaluation of the

benefit/risk balance of the use of depleting polyclonal antibodies in solid-organ transplantation and possibly reduce the incidence of PTLD.

Acknowledgments: We thank Mrs Marie-Noelle Marson for her technical skills. The authors are grateful to Laura Smales for copyediting.

Disclosure: The authors of this manuscript have no conflicts of interest to disclose as described by Nephrology – Dialysis and transplantation

The results presented in this paper have not been published previously in whole or part

References

1. Opelz G, Döhler B. Lymphomas after solid organ transplantation: a collaborative transplant study report. *Am J Transplant.* 2004; 4: 222

2. Bustami RT, Ojo AO, Wolfe RA, et al. Immunosuppression and the Risk of Post-Transplant Malignancy Among Cadaveric First Kidney Transplant Recipients. *Am J Transplant.* 2004; 4: 87

3. Faull RJ, Hollett P, McDonald SP. Lymphoproliferative Disease after Renal Transplantation in Australia and New Zealand. *Transplantation.* 2005; 80: 193

4. Caillard S, Lamy FX, Quelen C, et al. Epidemiology of Posttransplant Lymphoproliferative Disorders in Adult Kidney and Kidney Pancreas Recipients: Report of the French Registry and Analysis of Subgroups of Lymphomas: PTLD French Registry. *Am J Transplant.* 2012; 12: 682

5. Evens AM, David KA, Helenowski I, et al. Multicenter Analysis of 80 Solid Organ Transplantation Recipients With Post-Transplantation Lymphoproliferative Disease: Outcomes and Prognostic Factors in the Modern Era. *J Clin Oncol.*. 2010; 28: 1038

6. Trappe RU, Dierickx D, Zimmermann H, et al. Response to Rituximab Induction Is a Predictive Marker in B-Cell Post-Transplant Lymphoproliferative Disorder and Allows Successful Stratification Into Rituximab or R-CHOP Consolidation in an International, Prospective, Multicenter Phase II Trial. *J Clin Oncol.* 2017; 35: 536

7. Rabot N, Büchler M, Foucher Y, et al. CNI withdrawal for post-transplant lymphoproliferative disorders in kidney transplant is an independent risk factor for graft failure and mortality. *Transpl Int.* 2014; 27: 956

8. Dharnidharka VR, Webster AC, Martinez OM, et al. Post-transplant lymphoproliferative disorders. *Nat Rev Dis Primers.* 2016; 2: 15088

9. Sampaio MS, Cho YW, Shah T, Bunnapradist S, et al. Impact of Epstein-Barr virus donor and recipient serostatus on the incidence of post-transplant lymphoproliferative disorder in kidney transplant recipients. *Nephrol Dial Transplant.* 2012; 27: 2971

10. Francis A, Johnson DW, Teixeira-Pinto A, et al.. Incidence and predictors of post-transplant lymphoproliferative disease after kidney transplantation during adulthood and childhood: a registry study. *Nephrol Dial Transplant.* 2018; 33: 881

11. Cherikh WS, Kauffman HM, McBride MA, et al.. Association of the type of induction immunosuppression with posttransplant lymphoproliferative disorder, graft survival, and patient survival after primary kidney transplantation1. *Transplantation.* 2003; 76: 1289

12. Mueller T. Mechanisms of Action of Thymoglobulin. *Transplantation.* 2007; 84(Suppl 11): S5

13. Koene HR, Kleijer M, Algra J, et al. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. *Blood.* 1997; 3: 1109

14. Nagelkerke SQ, Schmidt DE, de Haas M, Kuijpers TW. Genetic Variation in Low-To-Medium-Affinity Fcgamma Receptors: Functional Consequences, Disease Associations, and Opportunities for Personalized Medicine. *Front Immunol.* 2019; 10: 2237

15. Cartron G, Dacheux L, Salles G, et al. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. *Blood.* 2002; 99: 754

16. Bibeau F, Lopez-Crapez E, Di Fiore F, et al. Impact of FcγRIIa-FcγRIIIa Polymorphisms and KRAS Mutations on the Clinical Outcome of Patients With Metastatic Colorectal Cancer Treated With Cetuximab Plus Irinotecan. *J Clin Oncol.* 2009; 27: 1122

17. Musolino A, Naldi N, Bortesi B, et al. Immunoglobulin G Fragment C Receptor Polymorphisms and Clinical Efficacy of Trastuzumab-Based Therapy in Patients With HER-2/neu –Positive Metastatic Breast Cancer. *J Clin Oncol.* 2008;26: 1789

18. Louis E, El Ghoul Z, Vermeire S, et al. Association between polymorphism in IgG Fc receptor IIIa coding gene and biological response to infliximab in Crohn's disease. *Aliment Pharmacol Ther*. 2004; 19: 511

19. Ternant D, Büchler M, Bénéton M, et al. Interindividual variability in the concentration-effect relationship of antilymphocyte globulins - a possible influence of FcgammaRIIIa genetic polymorphism. *Br J Clin Pharmacol.* 2008; 65: 60

20. Ternant D, Büchler M, Thibault G, Ohresser M, Watier H, Lebranchu Y, et al. Influence of FcγRIIIA genetic polymorphism on T-lymphocyte depletion induced by rabbit antithymocyte globulins in kidney transplant patients. Pharmacogenet Genomics. 2014; 24: 26

21. Stern M, Opelz G, Dohler B, et al.. Natural killer-cell receptor polymorphisms and posttransplantation non-Hodgkin lymphoma. *Blood.* 2010; 115: 3960

22. Matas AJ, Smith JM, Skeans MA, et al. OPTN/SRTR 2013 Annual Data Report: kidney. *Am J Transplant.* 2015; 15(Suppl 2): S1

23. Dall'Ozzo S, Andres C, Bardos P, et al. Rapid single-step FCGR3A genotyping based on SYBR Green I fluorescence in real-time multiplex allele-specific PCR. *J Immunol Methods.* 2003; 277: 185

24. Dall'Ozzo S, Tartas S, Paintaud G, et al. Rituximab-dependent cytotoxicity by natural killer cells: influence of FCGR3A polymorphism on the concentration-effect relationship. *Cancer Res.* 2004; 64: 4664

25. Zimmermann H, Weiland T, Nourse JP, et al. Fc[gamma]-Receptor IIIA Polymorphism p.158F Has No Negative Predictive Impact on Rituximab Therapy with and without Sequential Chemotherapy in CD20-Positive Posttransplant Lymphoproliferative Disorder. *J Immunol Res.* 2014; 2014: 1

26. Dharnidharka VR, Lamb KE, Gregg JA, et al. Associations between EBV serostatus and organ transplant type in PTLD risk: an analysis of the SRTR National Registry Data in the United States. *Am J Transplant*. 2012; 12: 976

27. Reshef R, Luskin MR, Kamoun M, et al. Association of HLA polymorphisms with post-transplant lymphoproliferative disorder in solid-organ transplant recipients. *Am J Transplant.* 2011; 11: 817

28. McAulay KA, Haque T, Crawford DH. Tumour necrosis factor gene polymorphism: a predictive factor for the development of post-transplant lymphoproliferative disease. *Br J Cancer*. 2009; 101: 1019

29. Akay E, Patel M, Conibear T, et al. Interleukin 28B gene polymorphisms and Epstein-Barr virus-associated lymphoproliferative diseases. *Intervirology.* 2014; 57: 112

30. Babel N, Vergopoulos A, Trappe RU, et al. Evidence for genetic susceptibility towards development of posttransplant lymphoproliferative disorder in solid organ recipients. *Transplantation.* 2007; 84: 387

31. Special Issue: KDIGO Clinical Practice Guideline for the Care of Kidney Transplant Recipients. *Am J Transplant.* 2009; 9(Suppl 3): S1

32. Franceschini E, Plessi J, Zona S, et al. Clinical Utility of Epstein-Barr Virus Viral Load Monitoring and Risk Factors for Posttransplant Lymphoproliferative Disorders After Kidney

Transplantation: A Single-Center, 10-Year Observational Cohort Study. *Transplant Direct.* 2017; 3: e182

33. Peters AC, Akinwumi MS, Cervera C, et al. The Changing Epidemiology of Posttransplant Lymphoproliferative Disorder in Adult Solid Organ Transplant Recipients Over 30 Years: A Single-center Experience. *Transplantation.* 2018; 102: 1553

34. Luskin MR, Heil DS, Tan KS, et al. The Impact of EBV Status on Characteristics and Outcomes of Posttransplantation Lymphoproliferative Disorder. *Am J Transplant.* 2015; 15: 2665

35. Morscio J, Dierickx D, Ferreiro JF, et al. Gene expression profiling reveals clear differences between EBV-positive and EBV-negative posttransplant lymphoproliferative disorders. *Am J Transplant.* 2013; 13: 1305

36. Karassa FB, Trikalinos TA, Ioannidis JPA. The FcγRIIIA-F158 allele is a risk factor for the development of lupus nephritis: A meta-analysis. *Kidney International.* 2003; 63: 1475

37. Brambila-Tapia AJL, Gámez-Nava JI, González-López L, et al. FCGR3A V(176) polymorphism for systemic lupus erythematosus susceptibility in Mexican population. *Rheumatol Int.* 2011;31: 1065

Figure legends

Figure 1: Flow chart (validation cohort).

From 139 paired of kidney recipients enrolled into a French multicentric matched case-control study, 111 were finally eligible to build our validation cohort.

Figure 2: PTLD-free survival by V158F polymorphism in patients from both cohorts who received T lymphocyte-depleting antibodies but no muromonab.

Patients were classified in two groups (VV and F-carriers, left panel) or three groups (VV, VF and FF recipients, right panel). Kaplan-Meier plots were compared with log rank test.

Figure 3: Effect of the V158F polymorphism on rabbit pIgG binding to FcγRIIIA expressed on NK-92 cells.

NK-92 cells transduced to express the FcγRIIIA-158V or the -158F allotype were incubated with a fixed concentration of FITC-conjugated anti-CD16 3G8 mAb, then varying concentrations of rabbit or human pIgG antibody for 30 min at 4°C for flow cytometry analysis.

Table 1: Kidney transplant recipient characteristics

	Discovery cohort (n=196)			Validation cohort (n=222)		
	PTLD (n=49)	No PTLD (n=147)	P-Value	PTLD (n=111)	No PTLD (n=111)	P-Value
Age at transplantation, years	44.5 [4.5-74.6]	44.9 [3.0-74.1]	0.492	49 [15-76]	49 [20-79]	0.477
Male recipient, number (%)	26 (53.1)	86 (58.5)	0.505	69 (62.2)	69 (62.2)	1
First transplantation, number (%)	45 (91.8)	137 (93.2)	0.753	99 (89.2)	98 (88.3)	0.832
Deceased donor, number (%)	48 (98.0)	145 (98.6)	1	.	.	.
Acute rejection, number (%)	15 (30.6)	53 (36.1)	0.488	29 (26.1)	30 (27.1)	0.879
CMV mismatch (D+R-)	14 (28.6)	44 (29.9)	0.857	.	.	.
Pretransplant recipients EBV seronegative[1]	5 (13.9)	4 (7.3)	0.474	15 (13.5)	13 (11.7)	0.686
HLA A, B, DR mismatches, number (%)[2]			0.333			0.263
- 0-1	3 (6.1)	8 (5.4)		9 (8.1)	9 (8.1)	
- 2-4	28 (57.1)	100 (68.0)		74 (66.7)	83 (74.8)	
- 5-6	18 (36.7)	39 (26.5)		24 (21.6)	15(13.5)	
Year of transplantation			0.908			0.717
- 1985-1994	23 (46.9)	68 (46.3)		28 (25.2)	25 (22.5)	
- 1995-2004	17 (34.7)	53 (36.1)		60 (54.1)	66 (59.5)	
- 2005-2014	9 (18.4)	26 (17.7)		23 (20.7)	20 (18.0))	
Basiliximab (%)	6 (12.2)	29 (19.7)	0.236	24 (21.6)	30 (27.0)	0.879
Lymphocyte depleting agents, number (%)						
- Polyclonal (rATG and/or ALG)[3]	36 (73.5)	111 (75.5)	0.775	73 (69.6%)	56 (54.9)	**0.030**
- Muromonab	5 (10.2)	2 (1.4)	**0.011**	2 (1.8%)	0 (0.0)	0.498
Initial maintenance immunosuppressive treatments, number (%)						
- Ciclosporine	38 (77.6)	122 (83.0)	0.394	78 (70.3)	80 (72.1)	0.767
- Tacrolimus	10 (20.4)	23 (15.7)	0.440	29 (26.1)	26 (23.4)	0.641
- Azathioprine	27 (55.1)	88 (59.9)	0.558	50 (45.0)	35 (31.5)	**0.038**
- Mycophenolate mofetil	21 (42.9)	60 (40.8)	0.802	57 (51.3)	71 (64.0)	**0.021**
- mTOR inibitors	0 (0.0)	1 (0.7)	1	3 (2.7)	5 (4.5)	1
- Steroids	147 (100)	147 (100)	1	108 (97.3)	109 (98.2)	0.722
Homozygous VV patients, number (%)	11 (22.4)	23 (15.6)	0.276	22 (19.8)	15 (13.5)	0.207

1: data missing in 105 patients from discovery cohort (PTLD: 13, No PTLD: 92); 2: data missing in 8 patients from validation cohort (PTLD: 4, No PTLD: 4); 3: 38 patients (PTLD: 13, No PTLD: 25) did not receive induction in validation cohort

D+, cytomegalovirus seropositive donor; R-, cytomegalovirus-seronegative recipient ; EBV, Ebstein Barr virus ; HLA: human leukocyte antigen ; mTOR, mammalian target of ramamycin

Table 2: Risk factors of PTLD in discovery cohort according to the induction therapy.

		HR	95% CI	P-value
All patients (n=196)				
	FCGRIIIA-158 VV (vs. F-carrier)	1.61	0.82-3.13	0.169
	T lymphocytes depleting agents (vs.no)	0.75	0.40-1.41	0.368
	Muromonab-CD3 (vs.no)	**5.22**	**2.07-13.19**	**0.0005**
	Recipient age (per year)	1.00	0.98-1.02	0.884
Patients treated by T lymphocytes depleting agents (rATG and/or ALG, n=147)				
	FCGRIIIA-158 VV (vs. F-carrier)	**2.08**	**1.01-4.35**	**0.046**
	Type of T lymphocytes depleting agents (vs. ALG)	0.97	0.67-1.39	0.853
	Duration of T lymphocytes depleting agents (per day)	0.97	0.88-1.06	0.453
	Muromonab-CD3 (vs.no)	**4.61**	**1.62-13.06**	**0.004**
	Recipient age (per year)	1.00	0.98-1.02	0.973
Patients treated by T lymphocytes depleting agents (rATG and/or ALG) without muromonab-CD3 (n=141)				
	FCGRIIIA-158 VV (vs. F-carrier)	**2.22**	**1.03-4.76**	**0.043**
	Type of T lymphocytes depleting agents (vs. ALG)	1.00	0.68-1.47	0.992
	Duration of T lymphocytes depleting agents (per year)	0.94	0.85-1.03	0.199
	Recipient age (per year)	1.00	0.98-1.02	0.897

Table 3: Risk of PTLD in homozygous VV kidney transplant recipients receiving T lymphocyte-depleting agents

	Validation cohort		
	HR	95% CI	P-value
Patients treated by T lymphocytes depleting agents (rATG and/or ALG) (n=129)			
FCGRIIIA-158 VV (vs. F-carrier)	1.69	0.97-2.94	0.0653
Muromonab-CD3 (vs.no)	**5.77**	**1.38-24.1**	**0.016**
Recipient age (per year)	1.01	0.99-1.02	0.593
Patients treated by T lymphocytes depleting agents (rATG and/or ALG) without muromonab-CD3 (n=127)			
FCGRIIIA-158 VV (vs. F-carrier)	**1.75**	**1.01-3.13**	**0.049**
Recipient age (per year)	1.00	0.99-1.02	0.562

Table 4. Comparison of post-transplant lymphoproliferative disorder (PTLD) characteristics between discovery and validation cohorts (A) and according to V158F polymorphism (B)

A	Discovery cohort (n=49)[1]	Validation cohort (n=111)[2]	P-Value
Early-onset PTLD, number (%)	6 (12.3)	24 (21.6)	0.161
Localization, number (%)			
- Gastrointestinal tract	12 (24.5)	21 (21.4)	0.675
- Lymph nodes	21 (42.9)	27 (27.6)	0.062
- Primary central nervous	11 (22.4)	9 (9.2)	**0.027**
- Graft	3 (6.1)	18 (18.4)	**0.049**
- Hematopoietic organs	5 (10.2)	11 (11.2)	0.851
- Other	12 (24.5)	23 (23.4)	0.891
Single site PTLD, number (%)	38 (77.6)	86 (73.0)	0.310
EBV-related, number (%)	26 (66.7)	41 (53.9)	0.190
Histologic data			
- B-cell PTLD	44 (93.6)	94 (95.9)	0.682
- T-cell PTLD	3 (6.1)	4 (4.1)	0.682

B

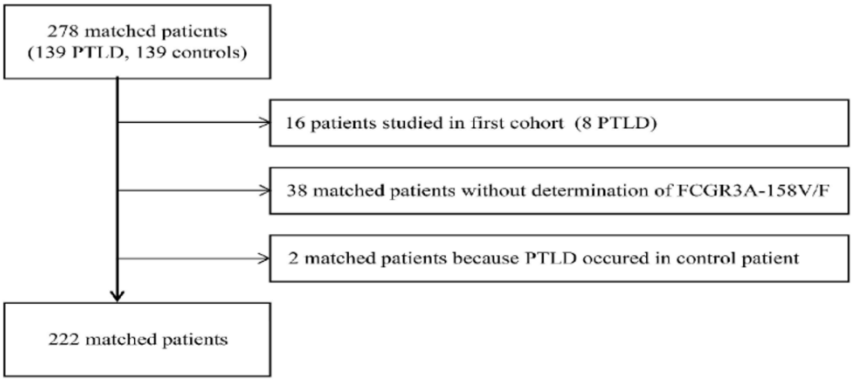
	Discovery cohort (n=49)[1]			Validation cohort (n=111)[2]			All patients (n=160)		
	F-carrier n=38	VV n=11	P-value	F-carrier n=89	VV n=22	P-value	F-carrier n=127	VV n=33	P-value
Early-onset, number (%)	5 (13.2)	1 (9.1)	1	22 (24.7)	2 (9.1)	0.151	27 (21.3)	3 (9.1)	0.137
Localization, number (%)									
- Gastrointestinal tract	10 (26.3)	2 (18.2)	0.708	19 (23.5)	2 (11.8)	0.352	29 (24.4)	4 (14.3)	0.319
- Lymph nodes	17 (44.7)	4 (36.4)	0.737	21 (25.9)	7 (41.1)	0.377	38 (31.9)	11 (39.3)	0.458
- Primary central nervous	9 (25.0)	2 (18.2)	1	9 (11.1)	0 (0.0)	0,352	18 (15.1)	2 (7.1)	0.367
- Graft	2 (5.3)	1 (9.1)	0.542	15 (18.5)	3 (17.6)	1	17 (14.3)	4 (14.3)	1
- Hematopoietic organs	3 (7.9)	2 (18.2)	0.311	9 (11.1)	2 (11.8)	1	12 (10.1)	4 (14.3)	0.508
- Others	10 (27.8)	2 (18.2)	0.708	21 (25.9)	4 (23.5)	0.938	31 (26.1)	6 (21.4)	0.612
Single site PTLD, numbr (%)	28 (73.7)	10 (90.9)	0.415	69 (83.1)	17 (89.5)	0.730	97 (80.2)	27 (90.0)	0.208
EBV- related, number (%)	21 (65.6)	5 (71.4)	1	35 (56.5)	6 (42.9)	0.389	56 (59.6)	11 (52.4)	0.546
B-cell PTLD[2]	35 (97.2)	9 (81.8)	0.132	76 (95.0)	18 (100.0)	1	111 (95.7)	27 (93.1)	0.561

1- Missing data: tumoral EBV status (n=10), histologic data (n=2)

2- Missing data: localization (n=13), number of sites (n=9), tumoral EBV status (n=35), histologic data (n=13)

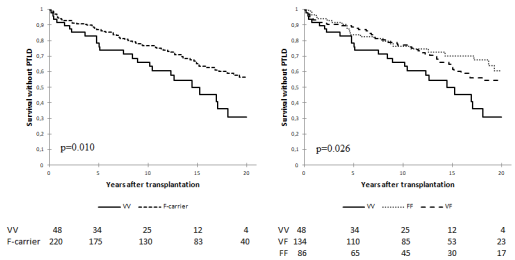
Early-onset: PTLD occuring durng the first post-transplant year

Figure 1



tri_13624_f1.tif

Figure 2

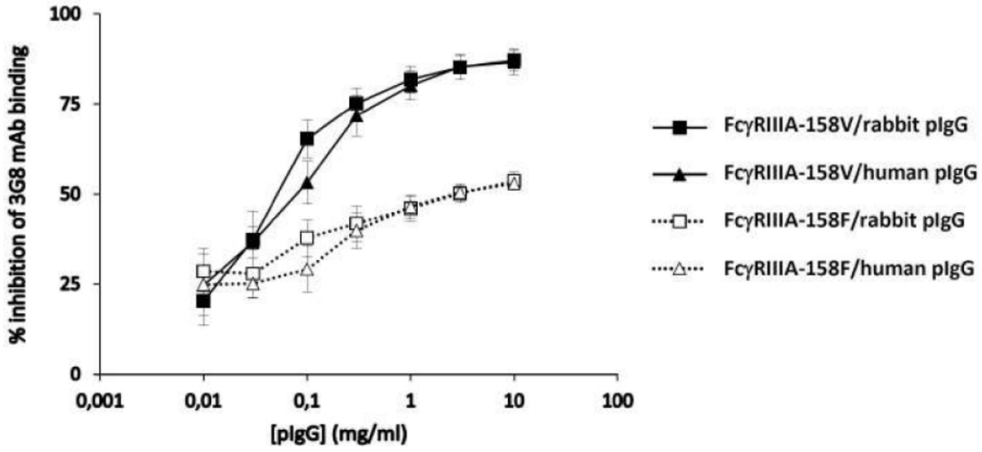


VV	48	34	25	12	4
F-carrier	220	175	130	83	40

VV	48	34	25	12	4
VF	134	110	85	53	23
FF	86	65	45	30	17

tri_13624_f2.tif

Figure 3



tri_13624_f3.jpg