Comparison of CMV ELISPOT and CMV Quantiferon™ interferon-γ releasing assays in assessing risk of CMV infection in kidney transplant recipients

Davide Abate¹*, Alda Saldan¹*, Carlo Mengoli¹, Marta Fiscon¹, Cristina Silvestre²,
Loredana Fallico¹, Marta Peracchi¹, Lucrezia Furian², Riccardo Cusinato¹, Luciana Bonfante¹, Barbara Rossi¹, Francesco Marchini², Dino Sgarabotto², Paolo Rigotti²,
Giorgio Palù¹

¹Department of Molecular Medicine - ²Department of Surgery, Oncology and Gastroenterology,
³Department of Medicine Nephrology I Unit, ⁴Hemodialysis and Nephrology II ⁵Transplant infectious disease division, Padua General Hospital - University of Padua School of Medicine via Gabelli, 63 - 35121 Padova Italy.

* Authors share equal contribution.

Keywords: Human Cytomegalovirus, transplantation, T-cell immunity, ELISPOT, Quantiferon

Running title: CMV ELISPOT and Quantiferon in kidney transplants

Abstract: 250 words
Text: 2270 words
Figures: 3
Tables: 4

Corresponding author:
Dr. Davide Abate
Department of Molecular Medicine
University of Padova
Via Gabelli, 63 35121 Padova Italy.
Phone: +39 0498218940
Fax: +39 0498216379
E-mail: davide.abate@unipd.it
Abstract

Background. Assessing the CMV specific cell-mediated immunity (CMI) represents an appealing strategy to detect transplant recipients at risk of infection. In this study we compared two interferon gamma-releasing assays (IGRAs), CMV Quantiferon and CMV ELISPOT, in predicting protective CMV specific T-cell responses.

Methods. 221 Quantiferon and ELISPOT tests were conducted on 120 adult KTR (including 100 R+ and 20 D+/R-). As control cohort 39 adult healthy subjects (including 33 CMV seropositive and 6 CMV seronegative) were enrolled. CMV IgG serology was used as reference for both tests.

Results. In CMV seropositive individuals, ELISPOT and Quantiferon provided 46% concordance with serology, 12% discordance, 18% disagreement between ELISPOT or Quantiferon with serology and 24% grey areas when one or both tests resulted weak positive. None of CMV seronegative subjects showed detectable responses in ELISPOT and Quantiferon. In transplant recipients, both ELISPOT and Quantiferon positively correlated with each other and negatively correlated to CMV DNAemia in a significant way (p-value <0.05). During the antiviral prophylaxis, all 20 D+/R- KTRs examined displayed undetectable Quantiferon and ELISPOT and there was no evidence of CMV seroconversion. The ROC statistical analysis revealed similar specificity and sensitivity in predicting detectable viremia (AUC 0.66 and 0.62 for Quantiferon and ELISPOT respectively). ELISPOT and Quantiferon values above 150 spots/200000 PBMCs and >1-6 IU IFN-g were associated with protection from CMV infection (OR 5 and 8.75 respectively).
Conclusion. In transplants both tests displayed similar ability in predicting CMV infection. Both ELISPOT and Quantiferon require several ameliorations to avoid false negative results.

Abbreviations: KTR, kidney transplant recipients; R+, CMV seropositive transplant recipient; D+/R-, CMV serogenative transplant recipient of a CMV positive donor; ROC, receiving operator characteristics; AUC, area under the curve. PBMCs, peripheral blood mononuclear cells; OR, odd ratio.

Introduction

Human cytomegalovirus (CMV) represents one of the primary opportunistic pathogen and a leading cause of morbidity and mortality in transplant subjects. The pre-emptive or prophylaxis antiviral therapy has effectively reduced the impact and incidence of symptomatic CMV infection, however the drug-related toxicity and the potential emergence of drug resistant CMV strains discourage a prolonged antiviral regimen. The cell mediated immunity (CMI), and in specific the CMV specific CD4+ and CD8+ T-cell response, is able to control viral replication and thus the onset of symptomatic infections (9, 11, 14, 16, 24). In CMV D+/R- transplants, de novo CMI develops upon primary CMV infection often originated from virus reactivation within the allograft, while in CMV R+ transplants CMI recovers from previously existing immunity. The assessment of CMV specific CMI has also been used to determine the individual risk of infection and as a helpful indicator in making therapeutic decisions such as whether initiate or terminate an antiviral treatment (1-4, 10, 13, 31). For this reason a diagnostic test assessing the status of immune reconstitution has been recommended in the current CMV management guidelines in transplant patients (17).
At the present, several methods are available to monitor the CMI in transplant recipients: several methods rely on the functional analysis of T-cell secreted cytokines or T-cell phenotype (ie IFN-\(\gamma\), TNF-\(\alpha\), IL-2, CD107a, PD-1) upon antigen stimulation, while other methods, such as tetramer assays are based on direct detection of antigen specific T-cells or cell proliferation assays (5, 27, 28, 32). Moreover tetramer assays are limited to few human leukocyte antigen (HLA) haplotypes and thus may not be applicable to all patients. Most of these methods rely on advanced technologies and costly reagents or require a long turnaround time-to-result that make the test of impractical use for clinical/diagnostic purposes. These issues and the lack of standardization have limited their use to highly specialized laboratories. In the recent years an increasing number of reports focused on IGRAs as diagnostic standard for detecting CMI towards infectious agents in humans (1-3, 7, 13, 18-20). IGRA tests provide a practical, standardized, rapid and cost-effective tool to assess the pathogen specific CMI (reviewed in (12, 21, 25). The most commonly used IGRAs, T-SPOT TB (ELISPOT) and TB-Gold (Quantiferon), were developed for detecting Mycobacterium tuberculosis (TB) responses. Both T-SPOT TB and TB-Gold are FDA approved tests for use in humans and display similar characteristics in assessing tuberculosis infection in immunocompetent subjects (6, 30). Some reports also support that T-SPOT TB may be useful in immunosuppressed population since it has higher sensitivity compared to TB-Gold (23, 26).

In this study we compared CMV IFN-\(\gamma\) ELISPOT and Quantiferon tests to assess their grade of agreement, correlation and ability to predict CMV infection. Both tests have been used in experimental settings to detect CMV specific T-cell responses in transplants (3, 8, 15, 18, 29, 34).
The main differences between CMV ELISPOT and Quantiferon include: 1) the stimulus peptide composition designed to stimulate selectively CD8+ T cells (Quantiferon) or both CD4+ and CD8+ T-cells (ELISPOT); 2) Quantiferon test evaluates the IFN-\(\gamma\) production in a volume of 1ml of whole blood, while ELISPOT considers the IFN-\(\gamma\) production in a given number of PBMCs isolated from blood; 3) Quantiferon quantitatively measures IFN-\(\gamma\) as international units (IU), while ELISPOT quantifies the spot forming colonies (SFC) produced by a given number of PBMCs;

Methods and Patients

Patients and definitions

221 ELISPOT and Quantiferon tests were performed on 125 KTRs enrolled in the study from September 2009 to September 2012. As control group we enrolled also 39 adult healthy subjects. The control group included 33 adult CMV IgG seropositive and 6 CMV IgG seronegative healthy subjects with median age 52 (range 24-72 years old), comprising 19 Caucasian male and 20 Caucasian female. 125 KTRs were voluntarily recruited among the transplants subjects at 30, 60, 90, 180 and 360 days after transplant. The main clinical characteristics of patients are shown in Table 1. Study exclusion criteria included any condition of pre-existing or acquired immunodeficiency. The Padua General hospital institutional review board and ethical committee approved all the medical and diagnostic procedures. CMV seropositive recipients (R+) were treated according to pre-emptive antiviral strategy once CMV DNAemia was detected >10,000 copies/ml of whole blood. CMV seronegative recipients of a CMV seropositive allograft (D+/R-) were treated according to antiviral prophylaxis regimen for 180 days after
transplant. Standard antiviral therapy comprised the administration of (val)-ganciclovir (Valcyte, Roche) at standard dose (900mg per day orally) corrected for renal functionality. No cases of CMV drug resistant strains occurred during the study. CMV disease is defined as fever, malaise, and/or gastrointestinal symptoms with concurrent CMV DNAemia and absence of other ongoing infections.

Detection of CMV viremia (DNAemia)

In all cases shown CMV viremia was evaluated using real time polymerase chain reaction (PCR) with an Abi Prism 7900 HT (Applied Biosystems). PCR primers, probes and PCR conditions were described (22). The lowest detection limit is defined as <1000 copies/ml of whole blood. CMV infection is defined as two sequential episodes of CMV DNAemia >1000 copies. Routine surveillance for viral reactivation or infection included weekly determination of CMV DNAemia during the first 100 days after transplant and continued thereafter if clinically indicated.

CMV serology

CMV serostatus was assessed using IgG ELISA assay (Enzygnost; Dade Behring). IgM ELISA (Enzygnost; Dade Behring) was used to detect primary CMV infection.

Quantiferon and ELISPOT tests

Both Quantiferon and ELISPOT were performed on freshly isolated blood: at the same time peripheral blood was collected in 3 (3x1ml) Quantiferon (Cellestis) tubes (Positive control, negative control-nil- and CMV stimulus) and 10 mls of peripheral blood were
collected in sodium citrate tubes for ELISPOT testing. Quantiferon blood tubes were incubated overnight at 37°C and further processed according to manufacturer’s instructions. Quantiferon data were acquired using Personal lab workstation (Adaltis). For ELISPOT testing, PBMCs were extracted using Ficoll-plaque Plus gradient (GE healthcare) and 200000 PBMCs/well were seeded in a 96 well ELISPOT plate (AID diagnostic) and stimulated as described (3). Quantiferon and ELISPOT tests were performed independently in double blind fashion. As indicated by manufacturer datasheet, ELISPOT was considered positive >20 spots, while Quantiferon >0.2 IU. The reported values refer to presence of CMI, not to protection from infection.

Statistical analysis

Stata software (StataCorp) was used to analyze the data. The correlation of ELISPOT to Quantiferon and CMV DNAemia was obtained by negative binomial regression where ELISPOT was expressed as number of spots, Quantiferon as IFN-γ cytokine concentration, and CMV DNAemia was a binary (0, 1) variable. ELISPOT and Quantiferon were evaluated by receiving operator curve (ROC) analysis, using as endpoint (reference variable) the protection against the emergence of an episode of detectable CMV viremia. Sensitivity and specificity were obtained for every possible cutoff of the ELISPOT or Quantiferon. The overall odds ratio (OR) was calculated as: sensitivity*specificity/[(1-sensitivity)*(1-specificity)]. We considered ELISPOT and Quantiferon levels protective if no detectable events of CMV DNAemia occurred within 60 days after ELISPOT and Quantiferon determination.
Results

Quantiferon and ELISPOT in respect to CMV IgG serology in healthy adult individuals

We compared the results of Quantiferon and ELISPOT in a cohort of 39 controls including 33 CMV IgG positive and 6 IgG negative subjects. None of these subjects resulted positive for CMV IgM. In 6/6 CMV IgG seronegative controls both Quantiferon and ELISPOT displayed undetectable values, in accordance with negative serology. Of the 33 IgG seropositive healthy subjects analyzed, 4/33 (12%) displayed undetectable CMI both with Quantiferon and ELISPOT, 15/33 (46%) resulted with positive CMI both for Quantiferon and ELISPOT (>40 spot and >0.3 IU IFN-γ), while in 6/33 (18%) ELISPOT and Quantiferon were discordant (4 ELISPOT +/ Quantiferon - and 2 ELISPOT - / Quantiferon +) and in 8/33 (24%) ELISPOT or Quantiferon displayed borderline weak positive value (table 2). The 4/33 (12%) subjects displaying undetectable CMI in Quantiferon and ELISPOT in disagreement with positive CMV IgG serology produced detectable high responses in ELISPOT when stimulated with whole CMV virion lysate (data not shown).

Quantiferon and ELISPOT in kidney transplants

A total 221 Quantiferon and ELISPOT tests were performed in a cohort of 120 kidney transplants including adult 100 CMV R+ and 20 CMV D+/R-. ELISPOT and Quantiferon analysis was performed at 30, 60, 90, 180 and 360 days after transplant.
In the group of 20 D+/R- all subjects analyzed displayed undetectable Quantiferon and ELISPOT and resulted with negative CMV IgG and IgM serology throughout the antiviral prophylaxis regimen.

In the group of 100 CMV R+ the time course of ELISPOT and Quantiferon revealed a consistent increase over time that peaked at 180 days after transplant, followed by a slight decrease at 360 days (figure 1).

Correlation of Quantiferon and ELISPOT and development of CMV DNAemia

In order to assess the correlation of Quantiferon and ELISPOT in transplants and the relationship of Quantiferon and ELISPOT with CMV DNAemia we employed a negative binomial regression statistical test. The results show that there is a statistical significant correlation between Quantiferon and ELISPOT ($p < 0.037$) and a statistical significant inverse correlation between Quantiferon and ELISPOT and development of CMV DNAemia ($p < 0.017$) (table 3 and figure 2). Since the exponentiated coefficient of Quantiferon is $10^{0.0740} = 1.076781$, a one-unit increase of Quantiferon predicts a 7.7% increase of ELISPOT.

We also attempted to correlate ELISPOT and Quantiferon with the magnitude (peak of viremia) and duration (days) of viremia using a linear regression approach but no significant difference was found (data not shown). The statistical analysis showed that the low number of cases might have caused the lack of significance.

Sensibility and specificity of Quantiferon and ELISPOT

In order to assess the sensitivity and specificity of Quantiferon and ELISPOT in preventing the onset of CMV DNAemia we employed the ROC statistical analysis (figure 3A-B). The calculated OR for ELISPOT was 2.12. The maximum OR value observed for
ELISPOT was 5.01, at cutoff values $\geq 147$. However, the OR mostly exceeded 4 in the cut-off area $\geq 119 - \geq 165$. The Quantiferon calculated OR was 2.52. The maximum value observed for OR was 8.75, at cutoff $\geq 6.1$. However, this cutoff is very high, and presumably not really useful, implying a low sensitivity of 19.13%. The ROC area, standard error and 95% confidence interval for Quantiferon and ELISPOT are reported in table 4. We also tested if the combining both ELISPOT and Quantiferon tests increases the ROC area. As shown in figure 3C, the combination of both tests results in a modest increase on the ROC area (0.67).

Discussion

Predicting the risk of infection in transplant recipients represents an innovative and promising strategy to improve the clinical management of transplant recipients. In this study we present the results of a comparative analysis of two IGRA tests, ELISPOT and Quantiferon, widely used to assess the CMI in transplants. In healthy CMV seronegative adult subjects both Quantiferon and ELISPOT were in agreement with the negative IgG serology, while in CMV IgG seropositive adults 12% of subjects resulted negative for both tests. This finding was unexpectedly high given previous reports (34) showing a 97% agreement with serology: the negative results of Quantiferon and ELISPOT opposing the positive CMV serology in healthy individuals may probably depend on previously shown inability of certain individuals in recognizing pp65 (ppUL83) stimulus peptide (33) or probably to atypical or non-common HLA haplotypes of these subjects (Abate et. al. submitted). Indeed CMV IgG+ individuals with negative CMI in Quantiferon and ELISPOT produced positive detectable high responses.
when stimulated with not-HLA restricted whole CMV virion lysate (data not shown), suggesting a limitation of the currently used stimuli in being recognized from heterogeneous HLA types. The consistency of the results from healthy subjects should be taken in account when immune monitoring is performed in transplants, in particular when a negative result is obtained. In transplant subjects we have found that none of the D+/R-receiving antiviral prophylaxis therapy resulted positive for Quantiferon, ELISPOT and CMV IgG or IgM. This finding suggests that the current antiviral prophylaxis scheme is highly effective in suppressing CMV reactivation from the allograft. This finding is also consistent with previously published data on D+/R- kidney transplants being unable to mount virus specific immune responses during the antiviral prophylaxis regimen (3). In transplants, Quantiferon and ELISPOT displayed a positive statistical significant correlation between the each other. Quantiferon and ELISPOT also showed a consistent increase over time peaking at 180 days after transplant, followed by a decrease at 360 days: this may suggest either a general slow process of immune reconstitution boosted by early post transplant episodes of CMV viremias, succeeding a steady state level of antiviral immunity.

In transplant subjects both Quantiferon and ELISPOT displayed similar robustness sensitivity and specificity and inverse correlation with development of CMV viremia suggesting that values >150 spots/200000 PBMCs for ELISPOT and >1-6 IU IFN-γ for Quantiferon may have good predictive value for protection from CMV viremia. The proposed cutoffs refer to protection from CMV viremia and are different from the cutoff of presence/absence of CMI proposed from the Quantiferon and ELISPOT manufacturers. This study to our knowledge is the first comparison of Quantiferon and
ELISPOT in transplants and the results from the present study may better aid clinicians to understand the limitations and the advantages of the two IGRA tests analyzed. This study has also elucidated some critical aspects that may be improved for both tests: there is an urgent need to overcome the unexpected high number of false negative results due to HLA type inability to recognize the CMV stimulus composition.

Acknowledgements
We are grateful to Padua General Hospital - Azienda Ospedaliera di Padova for providing us the IFN-γ ELISPOT plates and Cellestis and A.D.A, Italy, for providing Quantiferon and control tubes and reagents. We thank Daniel Tinto, Alice Bianchin, Angela Bozza, Simona Cofano and Valentina Formasiero for the technical guidance. We also want to thank Elisa Sefora Pierobon and Margherita Moro for medical advice.

Authors' contribution
DA, CM, DS, PR, GP analyze the statistical data and wrote the manuscript. AS, LF, MP, MF performed the tests and collected the experimental data. DA, LF, CS, PR, RC, LB, RB, MF, DS, CM, GP supervised the clinical study.

Conflict of interests
The authors declare no conflict of interest.
Figure and figure legend

Figure 1

A

B
Figure 1. ELISPOT (A) and Quantiferon (B) regression over time after transplant in CMV R+ subjects. Hollow circles represent single observations. A “Lowess smoother” (a locally weighted regression line, dashed line) was added for clarity.
Figure 2. The fitted values of ELISPOT score (predictions of the negative binomial model) are indicated as two lines, assuming an undetectable CMV DNAemia (dashed line) or a detectable CMV DNAemia (solid line). Hollow diamonds indicate the individual observations. 95% confidence intervals for the predictions are also indicated. ELISPOT values were limited to the scale 0–400, Quantiferon to 0-10.
Figure 3

A

Quantiferon

B

ELISPOT
Figure 3. ROC curve analysis using only (A) Quantiferon (black solid dots, AUC = 0.6604) or (B) ELISPOT (gray hollow dots, AUC = 0.6203) or (C) both tests in combination (AUC = 0.6731). The endpoint (reference variable) was the protection against the emergence of an episode of CMV viremia. The Quantiferon or ELISPOT scores were the classifying variables. Numbers on the curve represent the absolute Quantiferon or ELISPOT values.
### Table 1. Transplant characteristics

<table>
<thead>
<tr>
<th>Patient’s characteristics</th>
<th>KTRs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total n.</td>
<td>120</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>82 (71)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>38 (29)</td>
</tr>
<tr>
<td>Age (median, range)</td>
<td>38 (29-82)</td>
</tr>
<tr>
<td>CMV serostatus</td>
<td></td>
</tr>
<tr>
<td>D+/R+ and D-/R+ (%)</td>
<td>100 (83)</td>
</tr>
<tr>
<td>D+/R- (%)</td>
<td>20 (17)</td>
</tr>
<tr>
<td>Immunosuppressive regimen</td>
<td></td>
</tr>
<tr>
<td>CNI, MMF, and steroids (%)</td>
<td>120 (100)</td>
</tr>
<tr>
<td>Acute rejection episodes (%)</td>
<td>26 (22)</td>
</tr>
<tr>
<td>Patients who experienced post-transplant CMV DNAemia</td>
<td></td>
</tr>
<tr>
<td>All (%)</td>
<td>53 (44)</td>
</tr>
<tr>
<td>R+ (%)</td>
<td>47 (89)</td>
</tr>
<tr>
<td>R- (%)</td>
<td>6 (11)</td>
</tr>
<tr>
<td>Patients with CMV disease</td>
<td></td>
</tr>
<tr>
<td>All (%)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>R+ (%)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>R- (%)</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Patients who received treatment for CMV infection</td>
<td></td>
</tr>
<tr>
<td>All (%)</td>
<td>32 (27)</td>
</tr>
<tr>
<td>R+ (%)</td>
<td>27 (84)</td>
</tr>
<tr>
<td>R- (%)</td>
<td>5 (16)</td>
</tr>
</tbody>
</table>

Abbreviations: CNI, calcineurin inhibitors; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin.
Table 2. ELISPOT and Quantiferon test in CMV IgG+ individuals

<table>
<thead>
<tr>
<th>HS (%)</th>
<th>ELISPOT</th>
<th>Quantiferon</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 (46%)</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>4 (12%)</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>4 (12%)</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>2 (6%)</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>4 (12%)</td>
<td>Weak Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>1 (3%)</td>
<td>Weak Pos</td>
<td>Weak Pos</td>
</tr>
<tr>
<td>3 (9%)</td>
<td>Pos</td>
<td>Weak Pos</td>
</tr>
</tbody>
</table>

HS: healthy subjects; Positive values: ELISPOT >40, Quantiferon >0.3; Negative values: ELISPOT <20, Quantiferon <0.2; Weak positives: ELISPOT 20-40<; Quantiferon 0.2-0.3<. ELISPOT is expressed as number of spots/200.000 PBMCs; Quantiferon as IU IFN-g.
Table 3. Negative binomial regression of ELISPOT versus Quantiferon and CMV DNAemia in kidney transplants.

<table>
<thead>
<tr>
<th>ELISPOT</th>
<th>Coefficient</th>
<th>Std. Err</th>
<th>z</th>
<th>P</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>QUANTIFERON</td>
<td>0.0740</td>
<td>0.0355</td>
<td>2.09</td>
<td>0.037</td>
<td>0.0045</td>
</tr>
<tr>
<td>CMV DNAemia</td>
<td>-0.7020</td>
<td>0.2946</td>
<td>-2.38</td>
<td>0.017</td>
<td>-1.2794</td>
</tr>
<tr>
<td>incept</td>
<td>4.6125</td>
<td>0.1448</td>
<td>31.85</td>
<td>0.000</td>
<td>4.3287</td>
</tr>
<tr>
<td>Alpha</td>
<td>2.674709</td>
<td>0.2339</td>
<td></td>
<td></td>
<td>2.253408</td>
</tr>
</tbody>
</table>

Log likelihood = -1168.0423. LR chi2(2) = 11.00. Prob > chi2 = 0.0041

Table 4. ELISPOT and Quantiferon ROC curve method. The area under the curve (AUC) is reported, along with the standard error and the 95% confidence interval.

<table>
<thead>
<tr>
<th>ROC</th>
<th>Area</th>
<th>Std. Err</th>
<th>95% Conf. Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISPOT</td>
<td>0.6203</td>
<td>0.0458</td>
<td>0.53048</td>
</tr>
<tr>
<td>Quantiferon</td>
<td>0.6604</td>
<td>0.0447</td>
<td>0.57285</td>
</tr>
</tbody>
</table>
References


33. Sylwester, A. W., B. L. Mitchell, J. B. Edgar, C. Taormina, C. Pelte, F. Ruchti, P. R. Sleath, K. H. Grabstein, N. A. Hosken, F. Kern, J. A. Nelson,

Comparison of Cytomegalovirus (CMV) Enzyme-Linked Immunosorbent Spot and CMV Quantiferon Gamma Interferon-Releasing Assays in Assessing Risk of CMV Infection in Kidney Transplant Recipients

Davide Abate,\textsuperscript{a} Alda Saldan,\textsuperscript{a} Carlo Mengoli,\textsuperscript{a} Marta Fiscon,\textsuperscript{a} Cristina Silvestre,\textsuperscript{b} Loredana Fallico,\textsuperscript{a} Marta Peracchi,\textsuperscript{a} Lucrezia Furian,\textsuperscript{b} Riccardo Cusinato,\textsuperscript{a} Luciana Bonfante,\textsuperscript{c} Barbara Rossi,\textsuperscript{d} Francesco Marchini,\textsuperscript{d} Dino Sgarabotto,\textsuperscript{e} Paolo Rigotti,\textsuperscript{b} Giorgio Palù\textsuperscript{a}

Department of Molecular Medicine,\textsuperscript{a} Department of Surgery, Oncology and Gastroenterology,\textsuperscript{b} Department of Medicine Nephrology I Unit,\textsuperscript{c} Hemodialysis and Nephrology II Unit,\textsuperscript{c} and Transplant Infectious Disease Division,\textsuperscript{e} Padua General Hospital, University of Padua School of Medicine, Padua, Italy

Volume 51, no. 8, p. 2501–2507, 2013. Page 2502, Materials and Methods, “Patients and definitions” section, line 2: “125 kidney transplant recipients” should read “120 kidney transplant recipients.”

Page 2502, Materials and Methods, “Patients and definitions” section, line 7: “One hundred twenty-five KTRs” should read “One hundred twenty KTRs.”

Page 2502, Materials and Methods, “Patients and definitions” section: Information about the transplant immunosuppression induction protocol used was inadvertently omitted. The following statement should be inserted at the end of line 9: “The following induction immunosuppressive treatments were employed: antithymocyte globulin (ATG) for 67/120 patients (56\%) (median age of cohort, 60 years), basiliximab for 47/120 patients (39\%) (median age of cohort, 48 years), both ATG and basiliximab for 3/120 patients (2.5\%) (median age of cohort, 34 years), and alefacept for 3/120 patients (2.5\%) (median age of cohort, 33 years).” The overall results and conclusions of the study are unchanged.