Frequent Occurrence of Cytomegalovirus Retinitis During Immune Reconstitution Warrants Regular Ophthalmic Screening in High-Risk Pediatric Allogeneic Hematopoietic Stem Cell Transplant Recipients

Prashant Hiwarkar,1,4 Eva Gajdosova,3 Waseem Qasim,1,6 Austen Worth,1,6 Judith Breuer,5 Robert Chiesa,4 Deborah Ridout,1 Clive Edelsten,3 Anthony Moore,3 Persis Amrolia,1,4 Paul Veys,1,4 and Kanchan Rao4

1Molecular Immunology Unit and 2Centre for Pediatric Epidemiology and Biostatistics, Institute of Child Health, University College London; and Departments of 3Ophthalmology, 4Blood and Marrow Transplantation, 5Virology, and 6Immunology, Great Ormond Street Hospital for Children, London

Background. Although cytomegalovirus (CMV) retinitis (CMVR) is a well-recognized complication after allogeneic hematopoietic stem cell transplantation (HSCT), standard operating procedures for ophthalmic monitoring are variable. In particular, authors perceived a greater risk of CMVR after pediatric HSCT for inherited immunodeficiencies, in patients who often have pretransplantation viremia. This study was therefore performed to identify high-risk pediatric HSCT recipients who would benefit from regular ophthalmic monitoring.

Methods. During a 5-year study period, we retrospectively analyzed findings in 56 of 304 consecutive HSCT recipients (age range, 0.5–197 months) in whom significant CMV viremia developed (CMV level at PCR, ≥4000 copies/mL). All HSCT recipients with significant CMV viremia underwent retinal examination weekly (inpatients) or every other week (outpatients), with examinations performed by a skilled ophthalmologist.

Results. CMVR developed in 13 (4%) of 304 HSCT recipients, 23% (13 of 56) of those with significant CMV viremia. Pretransplant viremia (odds ratio, 11.3; P < .01), acute (grade ≥2) graft-vs-host disease (odds ratio, 8.2; P < .02) and mismatched graft (odds ratio, 8; P < .02) were identified as independent risk factors. Compared with other invasive CMV diseases, CMVR was more often a late-onset disease, occurring at a median of 199 days after HSCT. At diagnosis, a significantly higher CD4 T-cell count (≥200/µL; P < .03) and a lower CMV load (P < .001) was observed in children with CMVR, compared with those in whom lung, gut, or liver CMV disease developed.

Conclusions. We report an increased risk of CMVR in high-risk pediatric HSCT recipients. This form of CMV disease differs from other invasive CMV disease in its relationship to immune reconstitution and viral dynamics. We have studied the relationship between these variables and suggested a risk-stratified ophthalmic screening strategy.

Keywords. Cytomegalovirus (CMV); CMV retinitis, paediatric HSCT; ophthalmic screening.
to immune reconstitution inflammatory syndrome (IRIS), similar to that seen in the human immunodeficiency virus (HIV)–infected population [3]. In HIV infection, CMV IRIS is typically localized to the eye and presents in the form of vitritis, macular edema, and optic disc edema (immune recovery uveitis [IRU]), after initiation of highly active antiretroviral therapy (HAART). Because the risk of both CMVR and IRIS in the HIV-infected population is persistent, prolonged ophthalmic surveillance is now being recommended [4]. Similar ophthalmic surveillance may be indicated in high-risk pediatric HSCT recipients. This has considerable resource implications, because few cases of CMVR have been reported in pediatric HSCT recipients; ophthalmic screening programs are not well defined, and active surveillance is required because children do not reliably report the relevant symptoms [5, 6]. Therefore, in this retrospective study we aimed to (1) define the incidence of CMVR after pediatric HSCT, (2) examine the relationship between viral dynamics and immune reconstitution, and (3) devise a risk-stratified ophthalmic screening approach for pediatric HSCT recipients with CMV viremia.

METHODS

Study Population
We conducted a retrospective single-center study of 304 consecutive pediatric alloge neic HSCT recipients. During the study period, all patients were prospectively monitored for CMV viremia. Whole blood CMV polymerase chain reaction (PCR) monitoring was performed weekly until a CD4 T-cell count >300/µL was reached. CMV PCR assays were performed using Taqman probes on genomic DNA extracted from 200 µL of blood [7]. The sensitivity of the reaction was 100 CMV copies/mL. At our center, significant viremia was defined as >4000 copies/mL, and CMV-specific antiviral treatment was considered in such children [8]. The study included all children in whom CMV viremia developed (viral load, >4000 copies/mL).

Ophthalmic Monitoring
Dilated retinal examination was performed by a skilled ophthalmologist in all patients with significant viremia, weekly in inpatients and every other week in outpatients. Ophthalmic monitoring was continued until complete resolution of viremia.

Diagnosis of CMV Disease
End-organ CMV disease was diagnosed according to international definitions [9]. Recurrence of CMV disease was defined as manifestation that occurred after complete resolution of signs and completion of treatment. CMV disease occurring >100 days after HSCT was defined as late onset.

Flow Cytometric Analysis
At diagnosis of each episode of CMV disease, flow cytometry was performed using peripheral blood mononuclear cells (PBMCs) and FITC/PE-labeled antibodies against CD3, CD4, and CD8. When >2 million PBMCs could be isolated, these were stored for studying CMV-specific T-cell responses. During 15 such episodes, PBMCs were stored and enzyme-linked immunospot assay (ELISPOT) was performed to determine the frequency of CMV-specific interferon (IFN)–γ–producing cells, using anti-IFN-γ monoclonal antibodies (Mabtech), pp65 PepMix (1 µg/mL; JPT Peptide Technologies), and phytohemagglutinin (5 µg/mL; Roche Applied Science) with appropriate negative controls, as described elsewhere [10]. A positive response was defined as >20 spot-forming cells per 10⁶ cells above the background level.

Data Collection
Patient characteristics were identified from electronic database and case notes. These included age, sex, age at transplantation, indication for transplantation, conditioning regimen, graft source, and information on HLA matching, CMV serology, graft-vs-host disease (GVHD), corticosteroid treatment, onset and/or recurrence of CMV disease, and treatment for CMV disease (Table 1). GVHD was graded according to standard criteria.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients, No. (%) a</th>
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<tbody>
<tr>
<td>Age, median (range), mo</td>
<td>46 (0.5-197)</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>Primary immunodeficiency</td>
<td>27 (48)</td>
</tr>
<tr>
<td>Hematologic disorders (malignant or nonmalignant)</td>
<td>22 (39)</td>
</tr>
<tr>
<td>Metabolic</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Other immune disorders</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Pretransplant CMV viremia</td>
<td>19 (34)</td>
</tr>
<tr>
<td>Graft source</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>22 (39)</td>
</tr>
<tr>
<td>Peripheral blood stem cells</td>
<td>28 (50)</td>
</tr>
<tr>
<td>Cord blood</td>
<td>5 (9)</td>
</tr>
<tr>
<td>Bone marrow and peripheral blood stem cells</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Donor/recipient serologic status</td>
<td></td>
</tr>
<tr>
<td>Positive/positive</td>
<td>32 (57)</td>
</tr>
<tr>
<td>Positive/negative</td>
<td>10 (18)</td>
</tr>
<tr>
<td>Negative/positive</td>
<td>14 (25)</td>
</tr>
<tr>
<td>HLA-mismatched transplant</td>
<td>24 (43)</td>
</tr>
<tr>
<td>Anti-thymocyte globulin or alemtuzumab-based conditioning</td>
<td>40 (71)</td>
</tr>
<tr>
<td>Myeloablative conditioning (TBI or busulfan)</td>
<td>19 (34)</td>
</tr>
<tr>
<td>Acute GVHD</td>
<td>21 (38)</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>6 (11)</td>
</tr>
<tr>
<td>Corticosteroid treatment (1 mg/kg for &gt;8 wk)</td>
<td>17 (30)</td>
</tr>
</tbody>
</table>

Abbreviations: CMV, cytomegalovirus; GVHD, graft-vs-host disease; HLA, human leucocyte antigen; HSCT, hematopoietic stem cell transplant; TBI, total body irradiation.

* Data represent No. (%) of patients except where otherwise indicated.
Data on CMVR was retrieved from ophthalmic case notes, along with photographic evidence and hand drawings of the retina. CMV PCR data were retrieved from an electronic pathology reporting system.

**Statistical Analysis**

We compared CD4 T-cell counts (≥200 or <200/µL) and viral dynamics in patients with CMVR and nonretinitis disease, using χ² and unpaired t tests, respectively. We performed univariate logistic regression analysis, using potential predictive variables to identify significant risk factors for CMVR, and we used χ² and Wilcoxon signed rank tests to examine the relationship between these factors. We used risk factors at a cutoff of P ≤ .1 to generate a multivariable model, and we performed multiple logistic regression (backward-stepwise) analysis, using SPSS software, version 20.0 (Table 2).

**RESULTS**

**Incidence of CMV Disease in Pediatric HSCT Recipients**

A total of 304 patients underwent 321 HSCT procedures at our institution between February 2005 and December 2010. Significant CMV viremia requiring antiviral treatment was noted in 56 patients (18%). The median follow-up for this patient cohort was 62 months (range, 29–95). The patient characteristics for this cohort with significant viremia are outlined in Table 1. In 21 of 56 patients (38%) with significant viremia (7% of 304 HSCT recipients) 32 episodes of CMV disease occurred (20 episodes of retinitis, 12 of nonretinitis disease). CMVR was the most common CMV disease, occurring in 13 patients (4% of HSCT recipients and 23% with significant viremia), followed by pneumonitis in 7 patients. Gastrointestinal involvement developed in 3 patients, and hepatitis in 1. There were 7 recurrent episodes of retinitis and 1 recurrent episode of pneumonitis.

**Characteristics of CMVR**

Thirteen children had 18 episodes of active and 2 episodes of inactive CMVR diagnosed. Active CMVR was diagnosed at a median of 199 days (range, −9 to 541) after HSCT, compared with 77 days (range, −10 to 426) for nonretinitis diseases (P < .05; Figure 1A). Sixteen episodes of CMV disease occurred late (retinitis in 13, pneumonitis in 2, and colitis in 1). At diagnosis, ocular involvement was bilateral in 8 children, but all children subsequently had bilateral involvement. Children with CMVR did not routinely undergo CSF analysis. However, CNS symptoms occurred in 4 of 13 children after the diagnosis of CMVR. In these children, no CMV was detected at CSF CMV PCR analysis. In this cohort, CMVR predominantly occurred as a separate entity and, only 3 children with other CMV disease (tongue, colon, and pneumonitis) also had CMVR. The onset of CMVR in each of these children was >100 days after the onset of other CMV disease. At the time of diagnosis of nonretinal CMV disease, all 3 children had a CD4 T-cell count of <200/µL. In contrast, 2 of these 3 children had CD4 T-cell counts >200/µL when CMVR was diagnosed.

**Relationship of CMV Disease With Viral Dynamics and Immune Reconstitution**

Table 2. Risk Factors for CMV Retinitis

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Univariate Analysis</th>
<th>Multivariable Analysis b</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>P Value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age b</td>
<td>.04</td>
<td>0.3 (.09–.9)</td>
</tr>
<tr>
<td>Sex (male vs female)</td>
<td>.54</td>
<td>0.6 (1.2–2.3)</td>
</tr>
<tr>
<td>Pretransplant CMV viremia</td>
<td>0.02</td>
<td>5 (1.2–21)</td>
</tr>
<tr>
<td>CMV donor serologic status (positive vs negative)</td>
<td>.85</td>
<td>1.1 (.3–4.9)</td>
</tr>
<tr>
<td>Graft source (BM vs PBSCT vs CB)</td>
<td>.24</td>
<td>0.5 (1.1–1.5)</td>
</tr>
<tr>
<td>Conditioning intensity (myeloablative vs nonmyeloablative)</td>
<td>0.24</td>
<td>2.6 (.5–13.6)</td>
</tr>
<tr>
<td>HLA mismatch (10/10 vs &lt;10/10)</td>
<td>.10</td>
<td>2.7 (.7–9.6)</td>
</tr>
<tr>
<td>Acute GVHD (grade ≥2)</td>
<td>.04</td>
<td>3.6 (1–13)</td>
</tr>
<tr>
<td>Corticosteroid treatment (1 mg/kg for &gt;8 wk)</td>
<td>.47</td>
<td>1.6 (.4–5.9)</td>
</tr>
</tbody>
</table>

Abbreviations: BM, bone marrow; CB, cord blood; CI, confidence interval; CMV, cytomegalovirus; GVHD, graft-vs-host disease; HLA, human leucocyte antigen; NS, not significant; OR, odds ratio; PBSCT, peripheral blood stem cell transplant.

a The area under the receiver operating characteristic curve was 0.83 (P < .001).

b Age in days at transplantation was analyzed after log₁₀ transformation to normality.
disease. Although there was no statistically significant difference between peak CMV levels for the 2 groups, the median duration of significant viremia before the first diagnosed episode of CMVR was 137 days (range, 20–301) for patients with retinitis compared with 71 days (range, 0–150) for those with nonretinitis disease \((P < .02; \text{Figure 1B and 1C})\). In addition to the chronicity of viremia, significantly lower viral loads were noted at the diagnosis of CMVR compared with nonretinitis disease \((1.5 \times 10^4 \text{ copies/mL; } P < .004; \text{Figure 2})\). Peripheral CD4 T-cell counts of \(\geq 200/\mu L\) were observed during 8 episodes (44%) of active CMVR compared with only 1 episode (8%) of nonretinitis disease \((P < .03)\). In children with CD4 T-cell counts \(\geq 200/\mu L\), an expansion of CD8 T cells with concurrent low levels of viremia was noted (Figure 2). To investigate whether T-cell immune reconstitution occurred in parallel with CMV-specific immune responses, we performed IFN-\(\gamma\) enzyme-linked immunospot assays on 15 PBMC samples stored at diagnosis of CMV disease (10 from patients with CMVR, 5 from patients with nonretinitis CMV disease). Of these 15 samples, CMV-specific T-cell responses were observed in all children who had CD4 T-cell counts \(\geq 200/\mu L\) at the diagnosis of late CMV diseases (4 children with retinitis and 1 with pneumonitis).

### Description of CMV Retinitis

Based on ophthalmologic criteria, none of the 18 active episodes of CMVR were classified as typical IRU. Two episodes were classified as atypical immune reconstitution syndrome. One child presented with a bilateral anterior uveitis, posterior synechiae, and ciliary injection. These changes were found simultaneously with a granular retinitis and bilateral retinal detachments. At diagnosis of CMVR in this patient, the CD4 T-cell count was 210/\(\mu L\), and CMV load 2271 copies/mL. Another child presented with inflammatory vasculitic reaction in the form of frosted branch angiitis. In this patient, the CD4 T-cell count was 580/\(\mu L\), and the CMV load 608 copies/mL.

### Risk Factors for CMVR

Univariate analysis identified young age at HSCT, PID, pretransplant viremia, nonmyeloablative conditioning, and acute GVHD (grade \(\geq 2\)) as risk factors for CMVR. HLA mismatch \((P < .10)\) also satisfied the criteria for a multivariable model. We examined the relationship between these risk factors before multivariable modeling and found a significant association between young age at HSCT, PID, and pretransplant viremia \((P < .05)\). There was no significant relationship or collinearity between HLA mismatch and acute GVHD. In a multivariable model, acute GVHD (grade \(\geq 2\); odds ratio, 8.2; 95% confidence interval, 1.4–49.9), mismatched grafts (8.0; 1.2–52.8), and pretransplant viremia (11.3; 1.5–83.4) retained significance as independent risk factors. Although CMV-seronegative donor grafts did not increase the risk of CMVR, the incidence of CMV disease involving lung, gut, and liver was significantly higher in HSCT recipients receiving CMV-seronegative donor grafts \((P = .02)\).

### DISCUSSION

This is the first report of ophthalmic CMV disease in pediatric HSCT recipients in which viral monitoring and immune
surveillance was coupled with systematic ophthalmic screening of high-risk children. In this high-risk cohort, we found higher rates of CMVR (23%) than in a previous report on adults (4%) [13]. Similar to previous reports of CMVR in adult HSCT recipients, CMVR occurred late after HSCT in this pediatric cohort, after a period of chronic viremia [2, 13].

One explanation for CMVR after chronic viremia may be that the eye is a sanctuary site for CMV with poor access to systemic drugs. This could explain the occurrence of CMVR associated with low-level viremia. Another explanation could be that CMVR frequently presents during immune reconstitution, as seen in HIV-infected patients after initiation of HAART. Almost half the children in our study had CD4 T-cell counts >200/µL at the onset of CMVR. This immune reconstitution was observed to occur in parallel with CMV-specific IFN-γ responses. We also observed that although CMV diseases involving the lung, gut, or liver occurred more frequently when the donor was CMV seronegative, receipt of a CMV-seronegative donor graft did not increase the predisposition to CMVR. These observations suggest that in a subset of pediatric HSCT recipients, CMVR occurs after a prolonged period of viremia, during the phase of CMV-specific immune reconstitution. Therefore similar to findings in HIV infection, CMVR after HSCT may be an IRIS-like response observed after recovery of CMV-specific immunity in immunocompromised recipients, including those with PID.

In acquired immunodeficiency due to HIV, it is expected that one-third of patients with CMVR might develop overt inflammatory signs of IRU [3]. In our cohort, we did not observe the IRU typically described in the HIV-infected population. Two episodes with inflammatory signs were classified as atypical IRIS. One child with bilateral uveitis also had simultaneous granular retinitis and retinal detachments and hence could not be classified as having typical IRU. In another child, frosted branch angiitis developed, which has been anecdotally described to occur during the immune recovery phase after initiation of HAART in HIV infection [14]. Although the ocular signs of IRU are quite distinct from the various patterns of active and inactive CMVR, the diagnosis of mild IRU requires retinal imaging and slit-lamp examinations that are not feasible in many children. It is therefore possible that IRU may have been underestimated in our cohort, which includes young children.

Another important observation reported in the HIV-infected population is significant risk for IRU with a lower CD4 T-cell nadir before HAART; patients with IRU have been observed to have impaired CMV-specific CD4 T-cell responses compared

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**Figure 2.** Comparison of CD4 and CD8 T-cell counts and cytomegalovirus (CMV) levels (based on polymerase chain reaction analysis and plotted on a log scale) at diagnosis of retinitis and nonretinitis CMV disease. A correlation was observed between CD4 T-cell counts ≥ 200/µL, expanding CD8 T-cell counts and low CMV levels. There were significant differences in CD4 and CD8 T-cell counts and CMV levels between the CMV retinitis and nonretinitis CMV disease groups (P<.05). Symbols with dark centers correspond to positive CMV-specific T-cell responses. All patients with CD4 T-cell counts ≥200/µL in whom enzyme-linked immunospot assay could be performed had positive CMV-specific responses.
with those who had healed CMVR, suggesting that immune status before treatment confers greater risk of IRU than contemporary immune function [15]. Unlike that in HIV infection, the HSCT scenario is heterogeneous and complex, with immunity derived from surviving recipient cells as well as donor immune cells, both under the influence of pharmacologic immunosuppression for the prevention and/or treatment of GVHD or rejection. In our cohort, at the diagnosis of 15 of 18 episodes of active retinitis, some form of systemic immunosuppression was being administered. We therefore speculate that immunosuppression may have masked overt inflammatory signs and hence that retinal changes may not have been typical of IRU as seen in the HIV-infected population [16]. Most of the CMV IRIS experience comes from this population [17–22]. In HIV infection, treatment of IRU consists of systemic or local corticosteroid therapy [22,23]. Although the benefit of concomitant CMV-specific therapy (eg, valganciclovir) is unclear, most HIV clinicians prefer to use CMV-specific therapy in addition to steroids [24].

The success of intraocular and oral steroids depends on the severity of inflammation. Local steroids are very effective when the inflammation is mild, and patients with severe inflammation and macular edema often require aggressive treatment in the form of intravitreal steroids, which may reactivate CMV infection [22,23,25,26]. Therefore, in our cohort, in the absence of overt inflammatory reaction, reducing the antigenic burden of the virus by systemic and/or local treatment was chosen as a first-line treatment, and corticosteroids were considered only if there was evidence of IRU. Interestingly, 1 child in whom bilateral uveitis developed was not receiving any immunosuppression at the time of diagnosis and was the only child treated with steroids. The remaining 17 of 18 episodes of active retinitis were treated with systemic antiviral therapy and/or intraocular foscarnet. Fourteen of the 18 episodes were being treated with systemic antiviral therapy for CMV viremia at diagnosis of CMVR. For such episodes, treatment was further intensified with the addition of a second antiviral agent, with or without intraocular foscarnet.

At last follow-up, all 8 of 10 long-term survivors who could be evaluated had a degree of visual impairment, including 1 child with bilateral uveitis who experienced complete visual loss. These findings therefore have important implications for regular ophthalmic surveillance of high-risk pediatric HSCT recipients. In this pediatric cohort, preemptive CMV viremia and the previously described risk factors of acute GVHD (grade \( \geq 2 \)) and mismatched grafts were identified as independent risk factors [2,27]. Pretransplant CMV viremia is a frequent occurrence in children with a diagnosis of PID, who are also young at the time of HSCT. Fifteen of 19 children with pretransplant viremia had a diagnosis of PID. Although both PID and young age at HSCT were identified as risk factors at univariate analysis, in a multivariable model only pretransplant viremia retained significance. This suggests that though pre-transplant viremia is an independent risk factor, pretransplant viremia in children with PID may significantly increase the risk of CMVR.

Based on these findings, we have adopted an ophthalmic surveillance strategy for high-risk pediatric HSCT recipients. We commence ophthalmic screening in all pediatric HSCT recipients who have significant CMV viremia and any of the identified risk factors (ie, pretransplant viremia, PID, acute GVHD of grade \( \geq 2 \), or mismatched graft). Because CMVR was also observed to occur during cellular immune reconstitution when peripheral blood CMV levels were low, we continue ophthalmic surveillance in these high-risk pediatric HSCT recipients until complete resolution of CMV viremia, even if there is numerical evidence of T-cell recovery.

**Future Directions**

This study identifies potential risk factors for CMVR after HSCT. These risk factors are clinically relevant; however, the predictive power of this study is limited by a small cohort size. It is therefore important to confirm these findings in a prospective study in a large cohort of patients. This study also highlights that—in contrast to invasive CMV disease involving lung, gut, and liver, which occur mostly during lymphopenia—CMVR occurs during both the lymphopenic and lymphoid recovery phases. CMVR also occurs after a prolonged period of viremia, particularly when CMV levels are low. These striking differences between late CMVR occurring during immune reconstitution and other CMV diseases suggest that late CMVR occurring during the immune recovery phase may have be pathophysiologically distinct from other CMV diseases. However, in contrast to findings in HIV infection, the absence of typical IRIS-like findings in HSCT recipients warrants further study.

Detailed ophthalmic monitoring of high-risk pediatric HSCT recipients, including slit-lamp examination, may improve our understanding of CMVR occurring during immune reconstitution. Immune reconstitution monitoring, including T-regulatory analysis coupled with monitoring of CMV-specific immune responses, may also improve our understanding of immune system regulation after HSCT. In centers where expertise is available, viral culture and PCR analysis of aqueous humor fluid, particularly when intravitreal drugs are used to treat ophthalmic CMV disease, may provide meaningful insight into the pathophysiologic mechanism of ophthalmic CMV disease after HSCT.

**Notes**

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**Contributing authors.** P. H. performed research, analyzed the data, performed statistical analysis, and wrote the manuscript; E. G. performed...
research and analyzed the data and reviewed the manuscript; W. Q., J. B., R. C., C. E., A. M., P. A., and P. V. designed and performed research and reviewed the manuscript; A. W. and D. R. performed statistical analysis; and K.R. designed research and wrote and reviewed the manuscript.

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**References**


