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Review Article

Cytomegalovirus management after allogeneic hematopoietic stem cell transplantation: A mini-review

Chieh-Lin Jerry Teng [a,b,c], Po-Nan Wang [d], Yee-Chun Chen [e,f], Bor-Sheng Ko [g,h,*]

[a] *Division of Hematology/Medical Oncology, Department of Medicine, Taichung Veterans General Hospital, Taiwan*
[b] *Department of Life Science, Tunghai University, Taichung, Taiwan*
[c] *School of Medicine, Chung Shan Medical University, Taichung, Taiwan*
[d] *Division of Hematology, Department of Internal Medicine, Chang Gung Medical Foundation Linkou Branch, Taoyuan, Taiwan*
[e] *Division of Infectious Diseases, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan*
[f] *Department of Medicine, National Taiwan University, College of Medicine, Taiwan*
[g] *Division of Hematology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan*
[h] *Department of Hematological Oncology, National Taiwan University Cancer Center, Taipei, Taiwan*

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Abstract Because of the high incidence of cytomegalovirus (CMV) seropositivity in the population, CMV infection is a common and severe complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in Taiwan. Here we propose a CMV management strategy for patients undergoing allo-HSCT from the Taiwanese perspective, which focuses on the epidemiology, diagnosis, monitoring, prophylaxis, and treatment of CMV infection after allo-HSCT. In terms of CMV monitoring, weekly CMV monitoring with the COBAS® AmpliPrep system is the standard approach because the pp65 CMV antigenemia assay has a lower sensitivity than CMV monitoring with the COBAS® AmpliPrep system. However, pp65 CMV antigenemia assay has a better correlation with clinical symptoms in immunocompromised patients. A 14-week prophylactic course of letermovir is recommended for allo-HSCT recipients in Taiwan, especially for recipients of hematopoietic stem cells from mismatched unrelated and haploidentical donors. Preemptive ganciclovir therapy should be initiated when the CMV viral load exceeds 1000 copies/mL, and should not be discontinued until CMV DNA is no longer detected

* Corresponding author. Division of Hematology, Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan. Fax: +886 2 2956 2880.
E-mail address: kevinkomd@gmail.com (B.-S. Ko).

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in the blood. For allo-HSCT recipients who have CMV-related diseases, ganciclovir with or without CMV-specific intravenous immunoglobulin is the standard of care. The limited availability of foscarnet, an alternative for patients who are not responsive to or cannot tolerate ganciclovir, is a crucial issue in Taiwan. For pediatric allo-HSCT recipients, more data are needed to propose a CMV management recommendation.

Introduction

Cytomegalovirus (CMV) infection is a severe complication of allogeneic hematopoietic stem cell transplantation (allo-HSCT) that can cause various acute or late multiorgan diseases.[1] Despite advances in the diagnosis and management of CMV, CMV seropositivity remains a poor prognostic factor of non-relapse mortality for allo-HSCT recipients.[2] Because of the high incidence of CMV seropositivity in Taiwanese population, and more allo-HSCT are from human leukocyte antigen mismatched, or haploidentical donors, allo-HSCT-related CMV infection in Taiwan needs more attention. Here we reviewed the updated literature and developed CMV management recommendations for allo-HSCT patients from the Taiwanese perspective. In this review, we consider the epidemiology, diagnosis, monitoring, prophylaxis, and treatment of CMV infection after allo-HSCT.

Epidemiology

CMV seroprevalence in immunocompetent adults varies globally, from 40% to 100%.[3] Among the factors that can contribute to CMV reactivation in CMV seropositive individuals, allo-HSCT is an important cause. Advanced age is associated with a higher incidence of CMV antigenemia after allo-HSCT.[4] A proportion of patients with CMV reactivation eventually develop CMV-related diseases, among which pneumonitis is the most common and has a 70% mortality rate.[5]

In Taiwan, the adult CMV seropositive rate is as high as 90%, so CMV infection is a crucial issue in allo-HSCT.[6] With a cumulative probability of 48.7% at day 100 after allo-HSCT, CMV antigenemia was associated with inferior 1-year and 4-year overall survival (OS) in a consecutive Taiwanese cohort.[7] However, a study by Liu et al.[8] showed no statistical correlation between CMV infection and OS in allo-HSCT. They found that grade 2—4 acute graft-versus-host disease (GVHD) and anti-thymocyte globulin-containing conditioning regimens were associated with high CMV infection risks. Notably, the incidence of CMV infection in patients undergoing allo-HSCT in Taiwan is increasing, probably due to more profound immunosuppression during allo-HSCT than achieved with past regimens.[9]

Impact of donor and recipient CMV serostatus

Donor and recipient CMV serostatus have marked influences on survival after allo-HSCT. CMV immunoglobulin G (IgG)-positive (+) recipients of allografts from CMV seronegative donors have the worst outcomes among allo-HSCT recipients.[10] On the contrary, CMV-IgG-negative (−) recipients of allografts from CMV seronegative donors rarely develop severe CMV-related complications.[11] For patients with hematological diseases who are scheduled for allo-HSCT, CMV sero-status must be determined before allo-HSCT to avoid CMV sero-status contamination by passive transmission via CMV-IgG (+) blood product transfusion.[11] Notably, less than 30% of seronegative allo-HSCT recipients of sero-positive donor grafts develop CMV reactivation, whereas more than 80% of CMV sero-positive recipients are likely to develop CMV reactivation regardless of donor CMV sero-status.[12]

CMV reactivation and leukemia relapse

CMV reactivation is defined as a new episode of CMV antigenemia or CMV DNA in the blood (DNAemia) in patients who are CMV-IgG (+). Although an association between CMV reactivation and a lower likelihood of leukemia relapse in allo-HSCT has been thoroughly investigated, the results remain debatable. CMV replication seems to be a protective factor against acute myeloid leukemia relapse, but this potential benefit does not result in lower non-relapse mortality rates.[13,14] Further studies are needed to validate the protective effect of CMV infection on leukemia relapse.

CMV diseases

Unlike CMV infection diagnosis, the diagnosis of a CMV-related disease is more specific; it requires the detection of CMV in tissues by either molecular or virological methods in patients with CMV-related symptoms. CMV syndrome is diagnosed when patients have a fever ($\geq$38 °C) that lasts longer than two days and CMV is detected in their blood samples, but not in their tissues.[5] CMV retinitis differs from CMV gastrointestinal disease, myocarditis, pneumonitis, hepatitis, encephalitis/ventriculitis, and nephritis, which require evidence of CMV in the tissue along associated symptoms, in that CMV retinitis requires only appropriate ophthalmological clinical symptoms or signs for diagnosis.[15] Although more than 90% of adult HSCT recipients and donors in Taiwan are CMV seropositive, the overall incidence of CMV diseases after allo-HSCT is as low as 5%,[16] which may be due to the intensive CMV surveillance schedule and appropriate preemptive therapy after allo-HSCT. However, the mortality rate from CMV colitis can be as high as 20%.[17]

CMV monitoring

CMV monitoring methods

Intensive CMV viral load monitoring of allo-HSCT recipients with risk of CMV reactivation, by either molecular assays or the pp65 CMV antigenemia assay, may detect early CMV reactivation and facilitate the initiation of preemptive antiviral therapy. Compared with various in-house assays, the commercially available real-time quantitative polymerase chain reaction (qPCR) platform is recommended for monitoring due to its lower intra- and inter-assay variability.[18] Different commercial assays may assess the expression of different types and numbers of targeted genes, and use various probe types, DNA extraction platforms, PCR conditions, and subsequent analyses. Therefore, a consistent DNA extraction methodology, qPCR assay, and sample type are crucial for monitoring CMV DNAemia. Whole blood and plasma samples are equally robust for CMV DNAemia monitoring.[5] Importantly, the CMV DNA load values yielded by qPCR methods need to have a linear sensitivity range normalized to the World Health Organization (WHO) International Standard for CMV.[19]

The COBAS® AmpliPrep system (ROCHE) is the only commercial qPCR CMV DNA quantitative assay approved by the Taiwan Food and Drug Administration for the diagnosis, management, and monitoring of CMV infection in the setting of allo-HSCT.[20] Using plasma samples, the AmpliPrep system targets the CMV polymerase gene, and is calibrated to the WHO standard with a range from 137 to 9,100,000 IU/mL.[20]

The pp65 CMV antigenemia assay is an immunofluorescent staining method that detects pp65 antigens from the surface of CMV. The results of a pp65 CMV antigenemia assay may be affected by the use of different detection probes or antibodies, and by the neutrophil counts of whole blood samples. Although the pp65 CMV antigenemia assay has a lower sensitivity than qPCR, its results have a better correlation with clinical symptoms in immunocompromised patients.[21] Importantly, the pp65 CMV antigenemia assay has a shorter turnaround time and costs less than the qPCR CMV DNA quantitative assay. Some clinical laboratories in Taiwan still use the pp65 CMV antigenemia assay for CMV monitoring, but the qPCR CMV DNA quantitative assay has become the standard of care for precise CMV quantification.

CMV monitoring schedule

CMV DNAemia monitoring should be conducted at least once a week in the first 100 days after allo-HSCT. Patients with acute or chronic GVHD, prior CMV reactivation, or persistent T-cell immunosuppression or immunodeficiency may require an extended monitoring time.[22] More frequent CMV qPCR monitoring should be considered for CMV-seropositive recipients, CMV-seronegative recipients allografting from T cell-depleted, HLA-mismatched, or umbilical cord blood donors,[23] or patients with acute GVHD.[24]

CMV-specific CD8 T cells are crucial for CMV infection control. Interferon-γ produced by CMV-specific CD8 T cells is considered a marker of CMV protection.[25] CD8 T cell monitoring could be a practical approach to individualize CMV infection management.[5] However, the thresholds for CMV-specific CD8 T cell numbers that are sufficient for protection of allo-HSCT recipients from CMV syndrome or disease require validation. Currently, CMV surveillance guided by the number of CMV-specific CD8 T cells is not a routine CMV management strategy in Taiwan.

Preemptive treatment threshold

The timing of preemptive therapy initiation depends on a patient's risk of and potential for progression of CMV diseases. Both the European Conference on Infections in Leukaemia[5] and the recommendations and guidelines for the treatment of pneumonia in Taiwan[26] have proposed cutoffs for CMV DNAemia or antigenemia to guide preemptive antiviral therapy initiation. Compared with the pp65 CMV antigenemia assay, the plasma qPCR assays are more commonly recommended for CMV monitoring because the pp65 antigenemia assay is less sensitive in the neutropenic setting. The established cutoff for CMV DNAemia positivity is between 500 and 1000 copies/mL.[27] However, initiating preemptive antiviral therapy when the CMV viral load is less than 1000 copies/mL may be associated with shorter antiviral treatment duration.[28] More importantly, this preemptive strategy further reduces the risk of CMV diseases in allo-HSCT recipients.[29] Based on these data, most transplant centers in Taiwan consider a threshold of 1000–10,000 copies/mL to start CMV preemptive therapy after allo-HSCT.[9,30]

CMV management strategies

Prophylaxis

Previous studies have shown that high-dose acyclovir or valacyclovir prophylaxis markedly reduces CMV infection in allo-HSCT recipients. However, this approach does not further prevent the development of CMV diseases.[31,32] The efficacy of intravenous ganciclovir prophylaxis was also tested in the setting of allo-HSCT; although it provided no OS benefit, intravenous ganciclovir prophylaxis reduced the risk of CMV infection.[33] In addition to its unclear efficacy, ganciclovir prophylaxis is limited in the setting of allo-HSCT due to bone marrow suppression because approximately 50% patients would have developed ganciclovir-induced neutropenia.[34] Currently, ganciclovir is one of the strategies for CMV prophylaxis in allo-HSCT in Taiwan. However, the dose and preventive schedules are not conclusive.

A novel CMV terminase inhibitor, letermovir, has been approved for the primary prevention of CMV in sero-positive allo-HSCT recipients. It is approved by the Taiwan Food and Drug Administration, and has been the standard of care for CMV prophylaxis in allo-HSCT in Taiwan since December 2018. With a limited number of grade 3-4 adverse events, a 14-week letermovir course reduced CMV infection by 23.5% at week 24 after transplantation.[35] Although the all-cause mortality rate at week 48 was not significantly different between the letermovir and placebo groups (20.9% vs. 25.5%; $p = 0.12$),[35] a post hoc analysis notably indicated that letermovir might reduce mortality by preventing or

delaying clinically significant CMV infection in allo-HCT recipients. This result indicated that letermovir prophylaxis could reduce mortality in high-risk patients to a greater extent than in low-risk patients.[36] However, letermovir only targets CMV. Additional herpes simplex and varicella-zoster virus preventive agents must be used in combination with letermovir. Besides, drug—drug interaction between letermovir and calcineurin inhibitors or azoles needs more attention. Moreover, continuous CMV monitoring remains mandatory after prophylaxis completion because the incidence of CMV infection can increase at week 18 (Table 1).[35]

Preemptive therapy

Considering the potential toxicities of CMV prevention, the preemptive therapeutic approach guided by CMV viral load remains an important treatment strategy to control CMV infection. Ganciclovir is the most commonly used antiviral drug for CMV preemptive therapy. Preemptive ganciclovir in asymptomatic patients with CMV DNAemia or antigenemia markedly reduces the risk of CMV diseases.[29,37] Although pharmacokinetic studies have shown that oral valganciclovir can achieve comparable or even higher drug exposure than intravenous ganciclovir, the efficacy and safety of the two drugs are similar in CMV preemptive therapies.[38] A randomized trial demonstrated that foscarnet could be as effective as ganciclovir for CMV preemptive therapy,[39] but foscarnet is considered an alternative therapy for patients who have failed ganciclovir or valganciclovir treatment., Preemptive treatment concurrently with ganciclovir and foscarnet—which causes more adverse events than single-agent treatment—does not always yield better efficacy in allo-HSCT. A study by Mattes et al.[40] revealed that 17 (71%) of 24 bone marrow, liver, or renal transplantation recipients receiving preemptive ganciclovir therapy reached the primary end point of being CMV negative by PCR within 14 days compared with 12 (50%) of 24 patients receiving preemptive ganciclovir-plus-foscarnet ($p = 0.12$). Moreover, the toxicity level was greater in the combination-therapy arm. Cidofovir is a third-line CMV preemptive therapy.[41] However, its renal toxicity largely limits its clinical application. Data on leflunomide or artesunate in patients who failed other CMV preemptive therapies come from only a few case reports. In terms of the role of letermovir in the preemptive setting, a phase 2a study revealed promising results in renal transplant recipients.[42] However, its role in allo-HSCT remains unclear. Currently, only ganciclovir and valganciclovir are available and reimbursed in Taiwan for CMV treatment. Foscarnet is administered to only a minimal number of patients (Table 2).

Table 1 Phase 3 clinical studies of CMV primary prophylaxis in allo-HSCT.

	Study design	Number of patients	Main findings
Clinical trial			
Ljungman et al.[31]	Randomized, double-blind, acyclovir-controlled	1. Valacyclovir (n = 376) 2. Acyclovir (n = 372)	1. Valacyclovir was more effective than acyclovir in preventing or delaying CMV infection (HR for valacyclovir vs. acyclovir: 0.56; 95% CI: 0.45—0.71; $p < 0.0001$) 2. Survival did not differ between the valacyclovir and acyclovir groups (HR: 0.98; 95% CI: 0.73—1.31; $p = 0.89$)
Prentice et al.[32]	Randomized, double-blind, double-dummy	1. Intravenous acyclovir followed by oral acyclovir (n = 105) 2. Intravenous acyclovir followed by oral placebo (n = 103) 3. Low-dose oral acyclovir followed by placebo (n = 102)	1. Extending prophylaxis with oral acyclovir significantly improved survival at 7 months ($p = 0.012$) 2. The intravenous/oral acyclovir group and the intravenous/placebo group had similar survival ($p = 0.054$)
Winston et al.[33]	Randomized, placebo-controlled, double-blind	1. Ganciclovir (n = 40) 2. Placebo (n = 45)	1. The incidence of CMV infection was 20% in the ganciclovir group and 56% in the placebo group ($p < 0.001$) 2. The incidence of CMV disease was 10% in the ganciclovir group and 24% in the placebo group ($p = 0.09$)
Marty et al.[35]	Randomized, placebo-controlled, double-blind	1. Letermovir (n = 373) 2. Placebo (n = 195)	1. Clinically significant CMV infection occurred in 37.5% of patients in the letermovir group and 60.6% of patients in the placebo group by week 24 after allo-HSCT ($p < 0.001$) 2. All-cause mortality at week 48 was 20.9% among letermovir recipients and 25.5% among placebo recipients ($p = 0.12$)

CMV: cytomegalovirus; allo-HSCT: allogeneic hematopoietic stem cell transplantation; HR: hazard ratio; CI: confidence interval.

Table 2 Important clinical studies for preemptive CMV therapy in the setting of allo-HSCT.

	Study design	Number of patients	Main findings
Clinical trials			
Boeckh et al.[37]	Randomized, double-blind	1. Ganciclovirat engraftment (n = 112) 2. Antigenemia-guided ganciclovir (n = 114)	1. CMV disease developed in 14.7% of patients in the antigenemia-ganciclovir group and 2.7% of patients in the ganciclovir group before day 100 after allo-HSCT (p = 0.002) 2. The two groups of patients had comparable survival rates at day 100 (84% vs. 87%; p = 0.51), day 180 (73% vs. 71%; p = 0.91), and day 400 (61% vs. 59%; p = 0.80)
Heiden et al.[38]	Retrospective	1. Oral valganciclovir (n = 14) 2. Intravenous ganciclovir (n = 26)	1. Preemptive treatment with valganciclovir and ganciclovir led to similar CMV DNA viral load reduction (not significant)
Reusser et al.[39]	Prospective, randomized, open-label	1. Foscarnet (n = 110) 2. Ganciclovir (n = 103)	1. Event-free survival within 180 days after allo-SCT was 66% in the foscarnet group and 73% in the ganciclovir group (p = 0.6) 2. Retreatment for CMV infection after completion of preemptive therapy was required by 43% of the foscarnet group and 28% of the ganciclovir group (p = 0.06)
Bacigalupo et al.[52]	Retrospective	Preemptive therapy with foscarnet and ganciclovir combination (n = 32)	1. All patients cleared CMV antigenemia by day 15 2. Five patients had recurrent CMV antigenemia

CMV: cytomegalovirus; allo-HSCT: allogeneic hematopoietic stem cell transplantation.

Along with weekly CMV monitoring, preemptive therapy should be performed for at least two weeks. The treatment should not be discontinued until at least one set of negative CMV DNAemia or antigenemia results is obtained. In the first two weeks of preemptive antiviral therapy, an increasing CMV viral load may be due to host or drug-induced immunodeficiency, but not to resistance.[43] Treatment alteration is not mandatory in this scenario, and should be considered only if CMV DNA remains detectable after two weeks of treatment or the viral load increases by 10-fold after two weeks of preemptive therapy.[44] When the CMV viral load increases slowly, prolonged preemptive therapy with a full-dose regimen is feasible.

Treatment of CMV diseases

Ganciclovir is the standard of care for CMV diseases. Although not demonstrated by randomized controlled trials, a combination of intravenous ganciclovir and high-dose immunoglobulin is considered a treatment option for CMV pneumonitis.[45] The 2018 the recommendations and guidelines for the treatment of pneumonia in Taiwan suggests intravenous immunoglobulin or CMV-specific intravenous immunoglobulin combined with other antivirals to treat CMV pneumonitis after allo-HSCT.[26] The combination of immunoglobulin and antiviral agents to treat other CMV diseases remains debatable. For patients who experience ganciclovir-induced myelosuppression, granulocyte colony-stimulating factor could be a treatment option.[46] Although the 2018 recommendations and guidelines for the treatment of pneumonia in Taiwan also suggests both ganciclovir and foscarnet as a first-line treatment for CMV therapy in allo-HSCT patients, most Taiwanese patients are treated with ganciclovir because of the limited availability of foscarnet. The use of letermovir to treat CMV diseases remains limited by a lack of solid evidence.

Special considerations

Antiviral resistance

Antiviral drug resistance should be considered when the CMV viral load increases or symptoms of CMV diseases deteriorate while a patient is receiving an appropriate CMV treatment. CMV antiviral drug resistance is a rare clinical phenomenon, and usually occurs when allo-HSCT recipients have received appropriate treatments for several weeks. Although it is not routine clinical practice in most transplant centers in Taiwan, genotyping of antiviral resistance-associated mutations by DNA sequencing can be performed in some laboratories using in-house assays. Mutations in the CMV genes UL97 and UL54 are associated with ganciclovir resistance, and mutations in UL54 are associated with foscarnet and cidofovir resistance. Letermovir resistance is also commonly caused by UL56 mutations.[44] The optimal timing for analysis of CMV antiviral resistance has not been conclusively determined. However, surveys for CMV resistance should be conducted when patients are refractory to treatment, exhibit persistent viral load, or their viral load becomes elevated by more than 10-fold over the initial load. Deterioration of symptoms after appropriate treatment for more than two weeks is another factor to indicate CMV resistance surveillance.[44]

Pediatric patients

The CMV infection rate in pediatric patients undergoing allogeneic HSCT is higher than that in adult patients. Adult and pediatric patients, however, have comparable outcomes after CMV infection.[47] A study by Duver et al.[48] showed that 22.4% of pediatric allo-HSCT recipients could potentially develop CMV infections. Sero-positive pediatric recipients of seropositive donor allografts are the most vulnerable to CMV infection. Preemptive anti-CMV treatment with ganciclovir is encouraged in pediatric patients. However, the appropriate dose is an important issue that has not been conclusively determined. Research suggests that a half-dose of ganciclovir is appropriate for CMV preemptive therapy in pediatric allo-HSCT patients because the conventional dose of ganciclovir may result in a higher rate of severe neutropenia.[49] A Taiwanese study showed that 54 of 290 pediatric allo-HSCT recipients were diagnosed with CMV DNAemia (median CMV occurrence: day 76 after allo-HSCT). In addition, 44 of these 54 patients were CMV IgG (+) before allo-HSCT. Grade 3–4 acute GVHD, seronegative-donor-into-seropositive-recipient transplantation, and unrelated or mismatched donors were associated with incomplete preemptive therapy responses in this study.[30] Along with consideration of the toxicities and management burden of antiviral treatments, identification of risk factors for CMV reactivation is crucial in pediatric patients. Individualized preventive measures and monitoring strategies for pediatric allo-HSCT are recommended in Taiwan.

Secondary prophylaxis

For patients with CMV diseases within six months prior to allo-HSCT and a history of recurrent CMV infections,[50] secondary prophylaxis should be given upon completion of CMV disease treatment. In this clinical scenario, valganciclovir, valacyclovir, or letermovir would be the drugs of choice. Because of the unclear balance between the efficacy and toxicity of ganciclovir or valganciclovir, and the availability of alternative preemptive ganciclovir or valganciclovir, secondary CMV prophylaxis should be evaluated individually in Taiwan. Notably, letermovir administered as a secondary prophylactic agent may prevent CMV reactivation in a high-risk patient population.[51] Thus, secondary prophylaxis with letermovir could be considered a suitable option in the future.

Conclusion

CMV infection is a common and severe complication of allo-HSCT in Taiwan due to the high prevalence of CMV seropositivity. CMV monitoring at least once weekly using the COBAS AmpliPrep system is the standard approach. A 14-week prophylactic letermovir regimen is recommended for allo-HSCT recipients in Taiwan, especially for patients with mismatched unrelated and haploidentical donors. This approach reduces the incidence of CMV reactivation and all-cause mortality. However, drug–drug interaction between letermovir and calcineurin inhibitors or azoles needs more attention. Preemptive ganciclovir therapy should be initiated when the CMV viral load is higher than 1000 copies/mL, and should not be discontinued until CMV DNAemia is no longer detectable. For allo-HSCT recipients who suffer from CMV diseases, ganciclovir with or without CMV-specific intravenous immunoglobulin is the standard of care. Limited foscarnet availability in Taiwan is a pressing concern, as foscarnet is an alternative therapy for patients who fail to respond to or tolerate ganciclovir treatment. Additional data are needed to form a recommendation for CMV management in pediatric allo-HSCT recipients.

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Ethical approval

Not required.

Declaration of competing interest

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