New vaccines and antiviral drugs for cytomegalovirus

Paul Griffiths

Institute for Immunity & Transplantation, Royal Free Campus, UCL, London, NW3 2PF, United Kingdom

ABSTRACT

The natural history of cytomegalovirus (CMV) infection in transplant patients has been well established. This virus may originate from the recipient, the donor or both. When pre-transplant IgG antibodies in the recipient are taken into account, three types of infection are possible: primary, reactivation or reinfection. The risks of high viral load and end-organ disease are highest after primary infection and lowest after reactivation. Serial monitoring of patients by quantitative polymerase chain reaction for CMV DNA allows antiviral drugs to be deployed for pre-emptive therapy or an antiviral drug may be given prophylactically.

Both of these strategies are effective, but pre-emptive therapy has the advantage that randomised allocation of a new drug or placebo given prophylactically may show a reduced need for pre-emptive valganciclovir. In this review, I will consider what has been learned from use of ganciclovir and valganciclovir and apply this information to clinical trials that have evaluated maribavir, brincidofovir and letermovir.

In addition, pre-emptive therapy has the advantage of facilitating the discovery of vaccines against CMV using a pharmacodynamic approach. Briefly, patients awaiting transplantation are given vaccine or placebo pre-transplant. When they proceed to transplantation, various parameters of viral load can be compared to determine if the vaccine has an effect against CMV when compared to patients randomised to receive placebo. If there is evidence of control of CMV, this can be related to immune responses induced by the vaccine to define a correlate of protection. This review will summarise the published evidence available.

1. Introduction

The natural history of CMV infection after solid organ transplant is complex, with three different types of infection, depending on the pre-transplant serological status of the donor (D) or recipient (R) [1]. The D + R- combination means that primary infection in the recipient originates from the donor. The D–R + combination means that CMV reactivates from an infected recipient. The D + R + combination identifies patients at risk of either reactivation of latent virus or reinfection from the donor [2]. Cohorts of patients managed using pre-emptive therapy provide estimates of the frequency of these 3 types of infection. For example, our centre reported that patients undergoing transplantation of a kidney or liver had a 78% risk of primary infection and a 40% risk of reactivation. An intermediate value of 54% was found in the D + R + combination, leading to the estimate of 14% for the rate of reinfection if these patients are assumed to have the same 40% risk of reactivation as seen in the D–R + patients [1]. Ongoing viral replication allows the viral load to reach the high levels required to cause end-organ disease and the aim is to deploy antiviral drugs to prevent the viral load reaching high levels [3–5]. When considering which patients

have a viral load high enough to initiate treatment (3000 genomes/ml of whole blood, equivalent to 2520 international units/ml in our laboratory) then D + R- patients have the highest risk while D–R + patients have the lowest risk [1,6]. However, when considering the abundance of patients in each of the three subgroups, D + R + patients contribute most individuals who need to be treated [1]. As well as the proportion of people with viraemia, there are additional viral load parameters that can be assessed. The peak viral load seen post-transplant approximates to a normal distribution [1]. However, the distribution is shifted to the left in D + R + patients, showing that natural immunity is quite effective at controlling high viral loads. Natural immunity is, however, poor at controlling low viral loads as seen clearly in D–R + patients [1].

Ganciclovir was licensed in 1989 and valganciclovir in 2001. These drugs provided the mainstay for both pre-emptive therapy and prophylaxis, both of which can be used in solid organ transplant patients [7]. However, their bone marrow toxicity precludes use of these drugs for prophylaxis after stem cell transplant. This led to the policy of introducing an antiviral drug for prophylaxis only once the marrow had engrafted in an individual patient.

2. Newer drugs for CMV

Maribavir is an inhibitor of the protein product of gene UL97. Because ganciclovir is a substrate for the same protein, maribavir is contraindicated in patients who are also receiving ganciclovir [8].

Brincidofovir is a lipid prodrug of cidofovir that has much less renal toxicity than the parent compound. It also has better tissue distribution into multiple organs [9].

Letermovir inhibits the terminase complex encoded by CMV, which is a good target for antiviral chemotherapy because this enzymatic activity is not found in mammalian cells. The terminase complex cleaves concatameric DNA into the unit lengths that are each packaged into a preformed capsid within the nucleus of an infected cell [10].

3. Evaluation of these novel drugs at phase 2

All 3 drugs have been evaluated in stem cell transplant patients, where the epidemiology of CMV is less complex than in solid organ transplant patients. In nearly all cases, stem cell transplant patients reactivate latent CMV once they become immunocompromised, with only a small contribution from donor strains of CMV to cause primary infection [11].

The evaluation of all three drugs followed the pattern established previously for valganciclovir (Fig. 1). Once patients had engrafted, they were randomised to receive the new drug or a matching placebo for a duration of about 100 days. During this time, they were monitored by pre-emptive therapy as is the standard of care. Any patients with viraemia were then treated with ganciclovir or valganciclovir. The primary endpoint of the study was the ability of the novel drug to reduce the need for pre-emptive therapy measured at the time that prophylaxis stopped.

All three drugs met the phase 2 criteria and so proceeded to evaluation in phase 3 [9,10,12].

4. Evaluation of maribavir at phase 3

The design of the phase 3 study was changed from that used at phase 2. First, the primary endpoint was end-organ disease rather than the need for pre-emptive therapy. Second, the incidence of end-organ disease was assessed after a washout period following completion of prophylaxis. In addition, the lowest of the three doses of maribavir that had been evaluated in phase 2 was selected, rather than the maximum tolerated dose. [12] This study design was flawed because it allowed pre-emptive therapy to rescue patients from episodes of viraemia that had not been prevented by prophylaxis. It was also flawed, because pre-emptive therapy is nowadays so effective that end-organ disease has become so uncommon that it is an impractical endpoint for a clinical trial of reasonable size [13].

Maribavir failed its phase 3 evaluation [14].

5. Evaluation of brincidofovir at phase 3

The protocol was similar to that used for maribavir, except that clinicians were allowed to start therapy after transplant but before

Fig. 1. Diagram summarising evaluation of new drugs in phase 2 studies in stem cell transplant patients.

engraftment because of the lack of bone marrow toxicity seen in the phase 2 study. The study design was also improved, compared to that used for maribavir, by removing the need for end-organ disease as the primary endpoint. However, it still retained the washout period. In addition, another problem became apparent when it was seen that more cases of acute graft-versus-host disease were diagnosed in patients receiving the drug compared to those receiving placebo. Graft-versus-host disease is classically diagnosed by the triad of diarrhoea, rash and abnormal liver function tests. However, haematologists are keen to initiate steroid therapy as soon as possible if graft-versus-host disease is suspected clinically. Accordingly, some of them started steroid therapy in patients with diarrhoea without recognising that this side-effect can be produced by brincidofovir. The net result was that more patients randomised to receive the drug were given steroids than those who received placebo. This administration of steroids precipitated reactivation of CMV, which compensated for the ability of brincidofovir to suppress viraemia. The net result was that there was no overall significant reduction in the incidence of viraemia among those patients randomised to receive brincidofovir [15]. The suspicion that these clinical cases of graft-versus-host disease were misdiagnosed is supported by the observation that more of them are based on symptoms of diarrhoea alone in the patients allocated to drug rather than those given placebo [15].

Brincidofovir failed its phase 3 evaluation [15].

The lessons learned from this study are that physicians should be fully aware of the details of the protocol for evaluation of novel antiviral drugs including, in this case, a section describing how management of diarrhoea should have involved stopping administration of brincidofovir. These cases also illustrate the biological interaction between steroids and CMV and emphasise how this virus acts as an opportunist, taking advantage of any increase in the net state of immunosuppression.

6. Evaluation of letermovir at phase 3

The protocol was similar to that used for brincidofovir, except that clinicians were allowed to start therapy after transplant but before engraftment because of the lack of bone marrow toxicity seen in the phase 2 study [16].

The results in figure 2 of reference [16] showed a marked and significant reduction in the incidence of viraemia requiring initiation of pre-emptive therapy post-transplant. This difference persisted and remained significant after the washout period. These positive results show that prophylaxis can work for CMV where the drug is both safe and effective. Importantly, the results also show that measurements of viral load post-transplant remain informative even with a drug like letermovir which does not inhibit DNA replication.

The results of this study also prove that active infection with CMV contributes to overall mortality in these patients. The clinical trial measured all-cause mortality and reported that, as expected, the reduction in mortality was attributable to transplant -related mortality rather than relapse of the underlying haematological condition [16].

7. Lessons learned from these 3 phase 3 studies

The primary endpoint for new phase 3 studies should be the need for pre-emptive therapy. This is now based on extensive natural history data and the conclusion of a meta-analysis linking measures of CMV viral load with end-organ disease [3–5].

Additional features of the studies also need to be updated, because they are based on the historical precedent of evaluating ganciclovir. Specifically, where a drug has been shown in phase 2 to be without significant bone marrow toxicity, then it should be evaluated in patients soon after transplantation and before waiting for engraftment; a convenient time point is when patients are able to swallow oral medication.
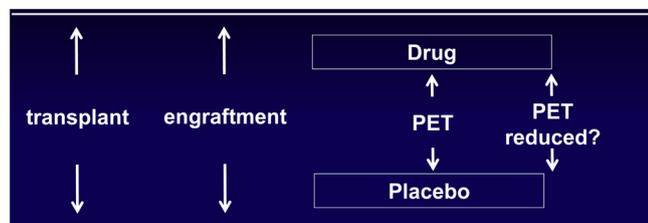
From what we know of the natural history of CMV, there is also no justification for maintaining a washout period after the end of prophylaxis. This is not done for evaluation of drugs for HIV, where it is recognised that the virus will rebound once treatment is stopped. In my opinion, the manufacturers who sponsor a clinical trial should define when it is safe for an individual patient to stop prophylaxis. For example, a test of cell-mediated immunity may be able to define a threshold value that indicates that an individual patient has a low risk of reactivation. Such evaluations should be built into future randomised controlled trial evaluations of novel antiviral drugs.

8. Evaluation of candidate vaccines in solid organ transplant patients

Three phase 2 studies have now been published. In 1984, Plotkin and colleagues gave the live attenuated Towne vaccine to seronegative patients awaiting renal transplantation [17]. Compared to recipients of placebo, the vaccine did not reduce the incidence of infection or end-organ disease, but did reduce the severity of disease. Measures of viral load were not available in those days but, because natural history studies show a strong correlation between a high viral load and the development of end-organ disease, it is very likely that this vaccine had its beneficial clinical effects by reducing viral loads post-transplant [3,4,18].

In 2011, our group reported that administration of a vaccine based on the CMV glycoprotein B plus MF59 adjuvant reduced parameters of viral load and the correlate of protection was the titre of antibodies made against glycoprotein B [19]. To follow up the interpretation that humoral immunity was important in these patients, I suggested to Genentech that seronegative recipients due to receive kidneys from seropositive donors should be randomised to receive an infusion of monoclonal antibodies specific for CMV or matching placebo. They organised a multicentre, multinational study with 120 D + R- patients and showed a significant reduction in post-transplant viraemia [20]. This proves that humoral immunity can reduce transmission of CMV from donor to recipient in sufficient quantities to cause viraemia post-transplant. As discussed elsewhere, additional studies will be required to determine if such interventions are potent enough to completely interrupt transmission of virus from donor to recipient [18]. This published study elevates the titre of antibodies from a correlate of protection to a mechanistic correlate of protection [21]. However, it must be acknowledged that the original observation of protection from antibodies was made against glycoprotein B whereas the infusion of monoclonal antibodies utilised those with activity against glycoprotein H and the protein derived from gene UL131, a component of the pentameric complex that is necessary and sufficient to allow CMV entry into endothelial cells [19,20].

Vincenti and colleagues in 2018 reported the administration of a plasmid vaccine including glycoprotein B and pp65, a major target of cell-mediated immunity, to seronegative renal transplant patients [22]. This study did not demonstrate immunogenicity of the vaccine or protection against viraemia. However, they did not give vaccine before transplant, but gave the first dose at 30 days after transplant [22].

The lessons to learn from this section should be to give vaccines pre-transplant before the patients receive immunosuppressive drugs. Administering vaccine at day 30 is far too late because, in natural history studies, 50% of D + R- patients have already developed viraemia by this time. [1] Furthermore, we know that CMV is transmitted from donor to recipient within hours of the transplant procedure [23].

9. Evaluation of candidate CMV vaccines in stem cell transplant patients

In 1986, Wimperis and colleagues gave tetanus toxoid or HBsAg vaccines to donors or recipients or both or neither to evaluate the possibility of adoptive transfer of immunity during stem cell transplantation [24]. The results showed that post-transplant antibody titres against each antigen were increased significantly when the vaccine was given to either the donor or the recipient compared to those who received placebo. The antibody titres were significantly increased again when the vaccine was given to both donor and recipient [24].

This concept was incorporated into the design of the evaluation of a CMV glycoprotein B and pp65 plasmid vaccine where three doses were given to donors of peripheral blood stem cells 2–21 days before donation [25]. Four doses were also given to seropositive recipients, one pre-transplant and then at one month, three months and six months post transplant. A total of 14 donor recipient pairs were studied before it was recognised that this part of the protocol was becoming impractical because of a move from using sibling donors to those with the best HLA matching identified through international registries. Because these patients came from multiple centres in many countries, it was not possible to immunise the donors pre-transplant. The study therefore continued by giving vaccine to another 80 individual recipients. The results showed a reduced need for pre-emptive therapy, with the number of ELISPOT cells against pp65 as a correlate of protection, and so this vaccine preparation proceeded to phase 3 [25]. It has recently been announced that this study failed to meet its primary endpoint. When the results are published in detail, it will be important to determine if the vaccine was immunogenic and/or whether the administration of vaccine to recipient only, rather than to the donor as well, could explain the differences between the results seen in phase 2 and phase 3.

10. Future studies

Substantial progress has been made with CMV by building on quantitative studies of the natural history of infection post transplant. In particular, the measures of viral load provided as part of the strategy of pre-emptive therapy are sufficiently robust to provide biomarkers that can be used in the evaluation of novel antiviral drugs and vaccines against this important pathogen.

It is likely that the focus of future studies will include combination antiviral therapy, comparison of prophylaxis with pre-emptive therapy and treatment of refractory or resistant infections. There may also be a debate about the relative merits of giving a drug with broad-spectrum activity against several different viruses versus a drug with specific activity against CMV alone. It will also be important to define how well combined approaches using vaccine pre-transplant plus antiviral drug post transplant can interact; the hope will be that some version of combination therapy can finally eliminate the problem of CMV post-transplant.

Competing interests

None.

The author has given advice to Chimerix and Shire and chaired the Data Safety Monitoring Board for the Phase 1 study of a vaccine made by Hookipa. In all cases, honoraria were paid to the author's institution, so he has no personal conflicts to report.

References

[1] S.F. Atabani, C. Smith, C. Atkinson, R.W. Aldridge, M. Rodriguez-Peralvarez, N. Rolando, et al., Cytomegalovirus replication kinetics in solid organ transplant recipients managed by preemptive therapy, Am. J. Transplant. 12 (9) (2012) 2457–2464.

[2] J.E. Grundy, S.F. Lui, M. Super, N.J. Berry, P. Sweny, O.N. Fernando, et al., Symptomatic cytomegalovirus infection in seropositive kidney recipients:

reinfection with donor virus rather than reactivation of recipient virus, Lancet 2 (8603) (1988) 132–135.

[3] A.V. Cope, C. Sabin, A. Burroughs, K. Rolles, P.D. Griffiths, V.C. Emery, Interrelationships among quantity of human cytomegalovirus (HCMV) DNA in blood, donor-recipient serostatus, and administration of methylprednisolone as risk factors for HCMV disease following liver transplantation, J. Infect. Dis. 176 (6) (1997) 1484–1490.

[4] J.M. McBride, D. Sheinson, J. Jiang, N. Lewin-Koh, B.G. Werner, J.K.L. Chow, et al., Correlation of cytomegalovirus (CMV) disease severity and mortality with CMV viral burden in CMV-Seropositive donor and CMV-Seronegative solid organ transplant recipients, Open Forum Infect. Dis. 6 (February(2)) (2019) ofz003 PubMed PMID: 30775403. Pubmed Central PMCID: 6366655.

[5] Y. Natori, A. Alghamdi, M. Tazari, V. Miller, S. Husain, T. Komatsu, et al., Use of viral load as a surrogate marker in clinical studies of cytomegalovirus in solid organ transplantation: a systematic review and meta-analysis, Clin. Infect. Dis. 66 (February(4)) (2018) 617–631 PubMed PMID: 29020339.

[6] P.D. Griffiths, E. Rothwell, M. Raza, S. Wilmore, T. Doyle, M. Harber, et al., Randomized controlled trials to define viral load thresholds for cytomegalovirus pre-emptive therapy, PLoS One 11 (9) (2016) e0163722 PubMed PMID: 27684379. Pubmed Central PMCID: 5042415.

[7] C.N. Kotton, D. Kumar, A.M. Caliendo, A. Asberg, S. Chou, L. Danziger-Isakov, et al., Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation, Transplantation 96 (August(4)) (2013) 333–360 PubMed PMID: 23896556.

[8] S. Chou, G.I. Marousek, Maribavir antagonizes the antiviral action of ganciclovir on human cytomegalovirus, Antimicrob. Agents Chemother. 50 (10) (2006) 3470–3472.

[9] F.M. Marty, D.J. Winston, S.D. Rowley, E. Vance, G.A. Papanicolaou, K.M. Mullane, et al., CMX001 to prevent cytomegalovirus disease in hematopoietic-cell transplantation, N. Engl. J. Med. 369 (13) (2013) 1227–1236.

[10] R.F. Chemaly, A.J. Ullmann, S. Stoelben, et al., Letermovir for cytomegalovirus prophylaxis in hematopoietic-cell transplantation, New. Engl. J. Med. 370 (2014) 1781–1789.

[11] E. Panagou, G. Zakout, J. Keshani, C. Smith, D. Irish, S. Mackinnon, et al., Cytomegalovirus pre-emptive therapy after hematopoietic stem cell transplantation in the era of real-time quantitative PCR: comparison with recipients of solid organ transplants, Transplant Infect. Dis. 18 (June (3)) (2016) 405–414 PubMed PMID: 27061703.

[12] D.J. Winston, J.A. Young, V. Pullarkat, G.A. Papanicolaou, R. Vij, E. Vance, et al., Maribavir prophylaxis for prevention of cytomegalovirus infection in allogeneic stem-cell transplant recipients: a multicenter, randomized, double-blind, placebo-controlled, dose-ranging study, Blood 111 (11) (2008) 5403–5410.

[13] F.M. Marty, M. Boeckh, Maribavir and human cytomegalovirus-what happened in the clinical trials and why might the drug have failed? Curr. Opin. Virol. 1 (6) (2011) 555–562.

[14] F.M. Marty, P. Ljungman, G.A. Papanicolaou, D.J. Winston, R.F. Chemaly, L. Strasfeld, et al., Maribavir prophylaxis for prevention of cytomegalovirus disease in recipients of allogeneic stem-cell transplants: a phase 3, double-blind, placebo-controlled, randomised trial, Lancet Infect. Dis. 11 (4) (2011) 284–292.

[15] F.M. Marty, D.J. Winston, R.F. Chemaly, K.M. Mullane, T.B. Shore, G.A. Papanicolaou, et al., A randomized, double-blind, placebo-controlled phase 3 trial of oral brincidofovir for cytomegalovirus prophylaxis in allogeneic hematopoietic cell transplantation, Biol. Blood Marrow Transplant. 25 (February (2)) (2019) 369–381 PubMed PMID: 30292744.

[16] F.M. Marty, P. Ljungman, R.F. Chemaly, J. Maertens, S.S. Dadwal, R.F. Duarte, et al., Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation, N. Engl. J. Med. 377 (December(25)) (2017) 2433–2444 PubMed PMID: 29211658.

[17] S.A. Plotkin, M.L. Smiley, H.M. Friedman, S.E. Starr, G.R. Fleisher, C. Wlodaver, et al., Towne-vaccine-induced prevention of cytomegalovirus disease after renal transplants, Lancet 1 (8376) (1984) 528–530.

[18] I.A. Baraniak, M.B. Reeves, P.D. Griffiths, Criteria to define interruption of transmission of human cytomegalovirus from organ donor to recipient, Rev. Med. Virol. 28 (January(1)) (2018) PubMed PMID: 29148608.

[19] P.D. Griffiths, A. Stanton, E. McCarrell, C. Smith, M. Osman, M. Harber, et al., Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial, Lancet 377 (9773) (2011) 1256–1263.

[20] J.H. Ishida, A. Patel, A.K. Mehta, P. Gatault, J.M. McBride, T. Burgess, et al., Phase 2 randomized, double-blind, placebo-controlled trial of RG7667, a combination monoclonal antibody, for prevention of cytomegalovirus infection in high-risk kidney transplant recipients, Antimicrob. Agents Chemother. 61 (February (2)) (2017) PubMed PMID: 27872061. Pubmed Central PMCID: 5278717.

[21] S.A. Plotkin, Complex correlates of protection after vaccination, Clin. Infect. Dis. 56 (May (10)) (2013) 1458–1465 PubMed PMID: 23386629.

[22] F. Vincenti, K. Budde, P. Merville, F. Shihab, V.R. Peddi, M. Shah, et al., A randomized, phase 2 study of ASP0113, a DNA-based vaccine, for the prevention of CMV in CMV-seronegative kidney transplant recipients receiving a kidney from a CMV-seropositive donor, Am. J. Transplant. (May 9) (2018) PubMed PMID: 29745007.

[23] H.A. Lumgair, N. Rolando, J. O'Beirne, D. Sharma, P.D. Griffiths, Transient residence of a seropositive organ is sufficient to transfer human cytomegalovirus to a seronegative recipient, Transplant Infect. Dis. 16 (June(3)) (2014) 501–504 PubMed PMID: 24666421.

[24] J.Z. Wimperis, M.K. Brenner, H.G. Prentice, J.E. Reittie, P. Karayiannis, P.D. Griffiths, et al., Transfer of a functioning humoral immune system in transplantation of T-lymphocyte-depleted bone marrow, Lancet 1 (8477) (1986) 339–343.

[25] M.A. Kharfan-Dabaja, M. Boeckh, M.B. Wilck, A.A. Langston, A.H. Chu, M.K. Wloch, et al., A novel therapeutic cytomegalovirus DNA vaccine in allogeneic haemopoietic stem-cell transplantation: a randomised, double-blind, placebo-controlled, phase 2 trial, Lancet Infect. Dis. 12 (4) (2012) 290–299.