

Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients: a phase 2 randomised placebo-controlled trial

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Summary

Background Cytomegalovirus end-organ disease can be prevented by giving ganciclovir when viraemia is detected in allograft recipients. Values of viral load correlate with development of end-organ disease and are moderated by pre-existing natural immunity. Our aim was to determine whether vaccine-induced immunity could do likewise.

Methods We undertook a phase-2 randomised placebo controlled trial in adults awaiting kidney or liver transplantation at the Royal Free Hospital, London, UK. Exclusion criteria were pregnancy, receipt of blood products (except albumin) in the previous 3 months, and simultaneous multiorgan transplantation. 70 patients seronegative and 70 seropositive for cytomegalovirus were randomly assigned from a scratch-off randomisation code in a 1:1 ratio to receive either cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant or placebo, each given at baseline, 1 month and 6 months later. If a patient was transplanted, no further vaccinations were given and serial blood samples were tested for cytomegalovirus DNA by real-time quantitative PCR (rtqPCR). Any patient with one blood sample containing more than 3000 cytomegalovirus genomes per mL received ganciclovir until two consecutive undetectable cytomegalovirus DNA measurements. Safety and immunogenicity were coprimary endpoints and were assessed by intention to treat in patients who received at least one dose of vaccine or placebo. This trial is registered with ClinicalTrials.gov, NCT00299260.

Findings 67 patients received vaccine and 73 placebo, all of whom were evaluable. Glycoprotein-B antibody titres were significantly increased in both seronegative (geometric mean titre 12537 (95% CI 6593–23840) versus 86 (63–118) in recipients of placebo recipients; $p < 0.0001$) and seropositive (118395; 64503–217272) versus 24682 (17909–34017); $p < 0.0001$) recipients of vaccine. In those who developed viraemia after transplantation, glycoprotein-B antibody titres correlated inversely with duration of viraemia ($p = 0.0022$). In the seronegative patients with seropositive donors, the duration of viraemia ($p = 0.0480$) and number of days of ganciclovir treatment ($p = 0.0287$) were reduced in vaccine recipients.

Interpretation Although cytomegalovirus disease occurs in the context of suppressed cell-mediated immunity post-transplantation, humoral immunity has a role in reduction of cytomegalovirus viraemia. Vaccines containing cytomegalovirus glycoprotein B merit further assessment in transplant recipients.

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Introduction

Cytomegalovirus is an important pathogen for women of childbearing age and for allograft recipients, two populations in whom development of a vaccine has been rated as high priority.^{1–3} The life-long latency and ability to reinfect despite pre-existing natural immunity make the production of a vaccine against cytomegalovirus challenging.^{4,5} In the allograft recipient, viraemic dissemination can cause end-organ disease, such as hepatitis, pneumonitis, gastroenteritis, and retinitis^{6,7} and can predispose to transplant rejection. The antiviral drug ganciclovir and its prodrug valganciclovir potently inhibit cytomegalovirus replication. Two strategies can be deployed to control end-organ disease related to the virus: antiviral prophylaxis, in which the drug is given

routinely from the time of transplantation; or pre-emptive treatment, in which patients are monitored to detect the virus in blood and treatment is begun once a defined quantity of viral load is detected. Both strategies are effective in control of such disease.^{8–13}

Cytomegalovirus infection after transplantation might originate from the donor or from reactivation in the recipient. Infection might cause either primary infection in recipients who are initially seronegative for the virus or reinfection with a new strain in seropositive recipients.⁴ The most serious clinical effects result from primary infection, followed by reinfection, with reactivation being the least likely to cause end-organ disease.⁴ Thus, most end-organ disease arises from donor-derived virus. This hierarchy of risk occurs because natural immunity before

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transplantation provides substantial protection against virus replication after transplantation^{14–16} and a high viral load is needed to cause end-organ disease.^{17–19}

Given that natural immunity before transplantation can modulate the pathogenicity of cytomegalovirus after transplantation,¹⁶ we tested whether vaccine-induced immunity could do likewise. No correlates of protective immunity that define whether a given vaccine is sufficiently immunogenic exist to justify a phase-3 clinical trial of efficacy. We therefore designed a phase-2 proof-of-concept study, selecting a group of patients given pre-emptive treatment as standard of care, so that no

patient received antiviral prophylaxis. This study focused on pharmacodynamics rather than pharmacokinetics.

Methods

Patients studied

In this phase-2 randomised placebo-controlled trial, patients were recruited from the kidney or liver transplant waiting lists at the Royal Free Hospital, London, UK, between Aug 3, 2006, and Oct 30, 2008. Exclusion criteria included: pregnancy (a negative pregnancy test was required before each vaccine dose); receipt of blood products (except albumin) in the previous 3 months, and

	Vaccine group		Placebo group	
	Cytomegalovirus positive	Cytomegalovirus negative	Cytomegalovirus positive	Cytomegalovirus negative
Total number of patients	32	35	38	35
Organ awaiting transplantation				
Liver	10 (31%)	15 (43%)	13 (34%)	16 (46%)
Kidney	22 (69%)	20 (57%)	25 (66%)	19 (54%)
Sex				
Male	16 (50%)	22 (63%)	17 (45%)	27 (77%)
Female	16 (50%)	13 (37%)	21 (55%)	8 (23%)
Age (years)	55 (12)	49 (12)	52 (12)	48 (13)
Race				
Caucasian	24 (75%)	32 (91%)	22 (58%)	33 (94%)
Black	1 (3%)	0 (0%)	7 (18%)	1 (3%)
Asian	5 (16%)	3 (9%)	5 (13%)	0 (0%)
Other	2 (6%)	0 (0%)	4 (11%)	1 (3%)
Number of vaccinations received				
1	1 (3%)	1 (3%)	4 (11%)	1 (3%)
2	12 (38%)	18 (51%)	12 (32%)	8 (23%)
3	19 (59%)	16 (46%)	22 (58%)	26 (74%)
Days from vaccine 1 to vaccine 2 (median, range)	32 (21–118) n=31	35 (22–274) n=34	30 (21–119) n=34	31 (23–241) n=34
Days from vaccine 1 to vaccine 3 (median, range)	186 (154–416) n=19	188 (147–224) n=16	188 (167–298) n=22	188 (151–375) n=26
Total number of patients who proceeded to transplantation during study period	18	23	22	15
Organ transplanted				
Liver	8 (44%)	11 (48%)	10 (46%)	10 (67%)
Kidney	10 (56%)	12 (52%)	12 (55%)	5 (33%)
Sex				
Male	7 (39%)	15 (65%)	7 (32%)	13 (87%)
Female	11 (61%)	8 (35%)	15 (68%)	2 (13%)
Age at transplantation (years)	53 (12)	50 (13)	50 (12)	49 (12)
Race				
Caucasian	12 (67%)	22 (96%)	14 (64%)	15 (100%)
Black	1 (6%)	0 (0%)	2 (9%)	0 (0%)
Asian	4 (22%)	1 (4%)	3 (14%)	0 (0%)
Other	1 (6%)	0 (0%)	3 (14%)	0 (0%)
Number of doses of vaccine or placebo received before transplantation				
1	0 (0%)	1 (4%)	1 (5%)	0 (0%)
2	9 (50%)	16 (70%)	8 (36%)	6 (40%)
3	9 (50%)	6 (26%)	13 (59%)	9 (60%)
Days from vaccine 1 to transplantation (median, range)	216 (40–636)	123 (22–604)	199 (8–1134)	262 (36–1231)

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	Vaccine group		Placebo group	
	Cytomegalovirus positive	Cytomegalovirus negative	Cytomegalovirus positive	Cytomegalovirus negative
(Continued from previous page)				
Immunosuppressive drugs administered*				
Basiliximab	5 (28%)	9 (39%)	7 (32%)	3 (20%)
Tacrolimus	14 (78%)	23 (100%)	22 (100%)	13 (87%)
Azathioprine	5 (28%)	6 (26%)	7 (32%)	4 (27%)
Mycophenolate mofetil	10 (56%)	17 (74%)	16 (73%)	10 (67%)
Prednisolone	13 (72%)	21 (91%)	19 (86%)	11 (73%)
Methylprednisolone	10 (56%)	16 (70%)	18 (82%)	9 (60%)
Available follow-up by PCR since transplantation (days)				
Median (range)	93 (89–228)	97 (15–138)	95 (45–173)	95 (20–278)
Cytomegalovirus status of donor				
Positive	7 (39%)	11 (48%)	15 (68%)	5 (33%)
Negative	11 (61%)	12 (52%)	7 (32%)	10 (67%)
Transplantation type				
Cadaver	13 (72%)	17 (74%)	16 (73%)	14 (93%)
Live person	5 (28%)	6 (26%)	6 (27%)	1 (7%)
Data are number (%), mean (SD) unless otherwise indicated. *If drugs known, otherwise we assumed that no drugs were administered.				
Table 1: Baseline characteristics according to patients' cytomegalovirus status and randomisation group				

simultaneous multiorgan transplantation. The study was approved by the Research Ethics Committee and all patients gave written informed consent.

Randomisation and masking

After patient consent, a pharmacist allocated placebo or vaccine using a scratch-off randomisation code provided by Sanofi Pasteur. The randomisation (ratio 1:1) was stratified by cytomegalovirus status (seropositive *vs* seronegative) and by transplanted organ (renal *vs* liver). Because the vaccine (white emulsion) and the placebo (colourless liquid) appeared different, a blind-observer procedure was followed for product preparation and administration and safety assessment. Specifically, one investigator prepared the vaccine by transferring 0.35 mL of the MF59 emulsion to the 0.35 mL of cytomegalovirus glycoprotein-B antigen vial and then withdrawing 0.5 mL to vaccinate the patient. A second investigator (unaware of whether vaccine or placebo had been given) was then responsible for safety assessment. The material to be injected was obscured from patients who were asked to face away from the injection site.

Procedures

The first patient was vaccinated on Aug 3, 2006. Vaccine doses containing 20 µg of recombinant cytomegalovirus glycoprotein B plus 9.75 mg of MF59 adjuvant were given intramuscularly at 0, 1, and 6 months, which was a dose schedule that had been previously assessed in healthy volunteers.^{20–22} The placebo was normal saline. Once a patient was transplanted, which could occur before all three doses were given, no further doses were

given. Patients received a diary card to record solicited local (injection site pain, erythema, and swelling) and systemic (headache, fever, and myalgia) symptoms for 7 days after every injection and a thermometer and ruler to help with these measurements. These adverse events were classified as mild, moderate, or severe by reference to a pre-specified chart. Patients were telephoned 48 h after every injection to remind them to complete the diary cards. Any serious adverse events that occurred within 28 days after an injection were recorded, and so were unexpected serious adverse reactions occurring at any time until the trial ended in September, 2009.

The principal investigator was responsible for study design, protocol development and, together with the statistician, prepared the prespecified data analysis plan. Adverse and serious adverse events were tabulated and presented to a Data Safety Committee on six occasions. This Committee felt it was necessary to break the code in April, 2009, to ensure that there was no imbalance in the number of deaths occurring in each study group, but all investigators remained unaware of the allocation until the formal breaking of the code in September, 2009, after all participants had completed the vaccination phase of the study.

Antibodies against glycoprotein B were measured by enzyme immunoassay with a method similar to that described in detail elsewhere²³ and expressed as geometric mean titres. Patients were managed according to routine clinical standard of care at this institute. Whole blood samples were requested twice a week from inpatients and at all subsequent outpatient visits, which were typically scheduled every week for 4 weeks then every 2 weeks until day 90. If cytomegalovirus viraemia was detected, patients

were monitored closely until PCR-negative results were obtained, and testing reverted to twice weekly in inpatients and at all subsequent outpatient visits. Each sample was tested by real-time quantitative PCR (rtqPCR) for cytomegalovirus DNA as described elsewhere.²⁴

Viraemia was defined as a blood sample that was PCR positive (cutoff 200 genomes per mL whole blood). If viraemia higher than 3000 genomes per mL was detected, the patient was treated with twice daily intravenous ganciclovir 5 mg/kg (or twice daily oral valganciclovir 900 mg), with dose adjustment for renal function, until cytomegalovirus DNA was undetectable in two consecutive blood samples. The time from the first PCR-positive sample until the last PCR-positive sample defined duration of viraemia, which therefore included days with and without pre-emptive treatment. Previous comparisons showed that changes in viral load values were indistinguishable when patients were treated with ganciclovir or valganciclovir.²⁴ Cytomegalovirus end-organ disease was diagnosed by histopathological demonstration of inclusion bodies in affected organs²⁵ and, from our natural history data,²⁶ was associated with a median viral load of 175 500 genomes per mL in blood with 37 000 genomes per mL as the lower limit of the 95% CI. With the 1 day average doubling-time^{14,15} of cytomegalovirus and the timing of sampling twice weekly, we aimed to initiate pre-emptive treatment once the viral load increased above 3000 genomes per mL to prevent viral load reaching 37 000 genomes per mL.

For the studies of immunogenicity, serum samples were requested at the time of first injection and 28, 56, 180, and 208 days later in those who received all three injections. In the subset of patients who received transplants, additional samples were requested at time of transplantation and 7, 35, 63, and 90 days later. The geometric mean titre and 95% CI of antibodies measured against glycoprotein B was calculated at each timepoint and plotted according to patient cytomegalovirus serostatus and randomisation group. Neutralising antibodies were measured with Towne RC256 (β -galactosidase marker virus) and human fibroblast target cells.

Safety and immunogenicity were co-primary endpoints. For secondary endpoints, we postulated that receipt of vaccine would decrease the duration or quantity, or both, of viraemia when compared with that of placebo. A correlate of protective immunity was the tertiary endpoint.

Statistical analysis

Safety and immunogenicity was assessed by intention to treat in patients who received at least one dose of vaccine or placebo (intention to treat-exposed analysis). No interim analyses were planned and no post-hoc analyses are presented. The percentage of patients reporting any pain (regardless of severity) within a week of first injection was compared in the two groups with a χ^2 test. Tests were two-sided and a p value of less than 0.05 was regarded as significant. Patients who did not complete a diary card

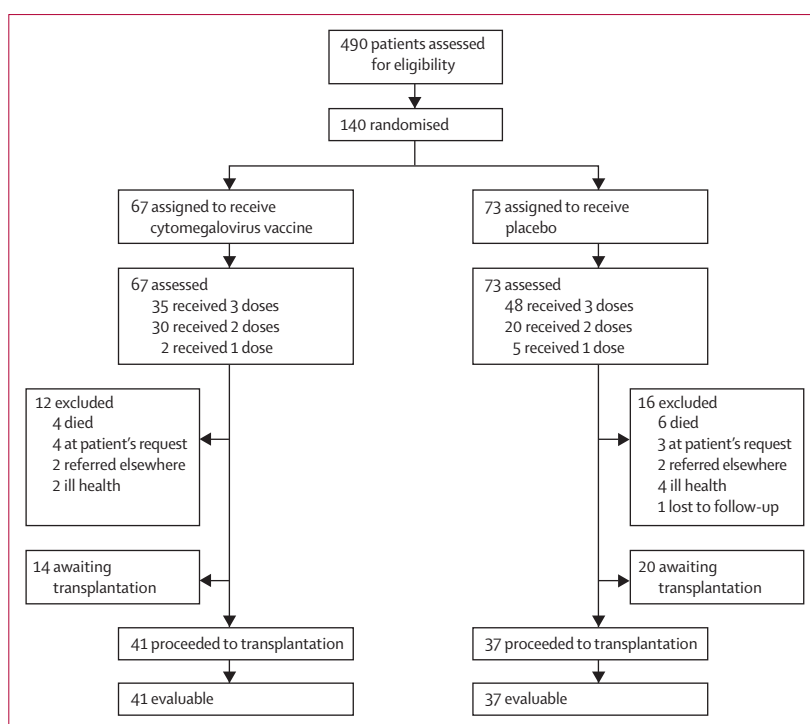


Figure 1: Trial profile at the time of analysis

were judged to have had pain (missing-equals-failure analysis). This analysis was repeated for the occurrence of other solicited adverse events (myalgia, redness, site swelling, headaches, or fever) within 1 week of the first injection. The missing-equals-failure analysis was repeated for the percentage of patients reporting pain within a week of the second and third doses, but excluding those who proceeded to transplantation since this was a random event (modified missing-equals-failure approach). Next, the results from all three injections were summarised descriptively according to severity (and thus each individual could have up to three measurements reported for each adverse event). Those who did not complete a diary card were excluded from the combined analysis of degree of severity.

For the studies of immunogenicity, because the outcome was numerical, a missing-equals-excluded approach was taken. The main timepoint of interest was decided a priori to be 1 month after the second dose of vaccine (day 56) and differences between groups were compared by a 2-sample *t* test (with log-transformed data to ensure normality) stratified by the patients' cytomegalovirus status. The investigation was powered so that about 30 patients with cytomegalovirus viraemia after transplantation would be expected, on the basis of our previous natural history data.¹⁶ A total sample size of 140 patients was required to expect about 30 patients to develop viraemia. Analyses were done with SAS version 9.2 (SAS Institute Inc, Cary, NC). This trial is registered with ClinicalTrials.gov, NCT00299260.

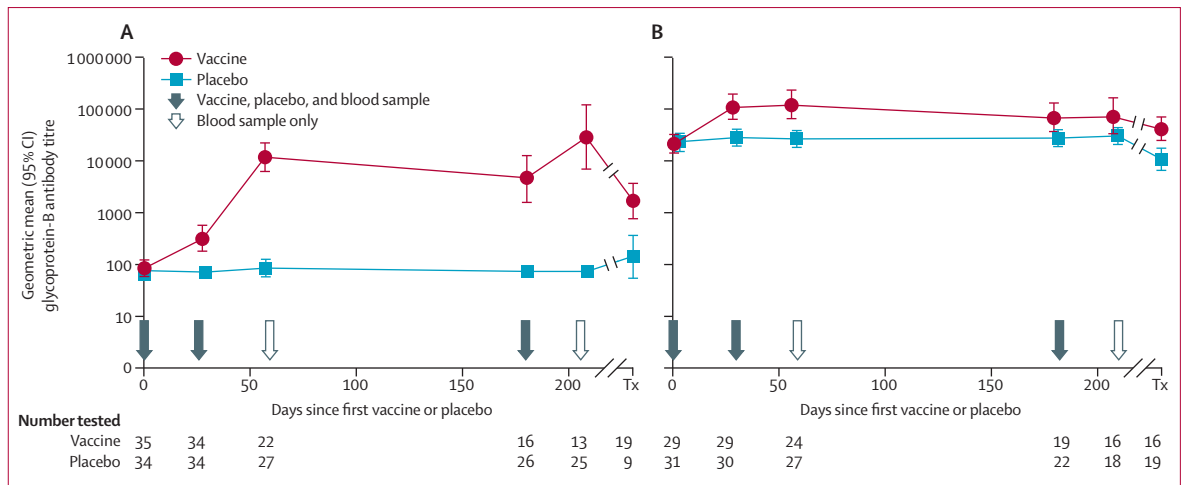


Figure 2: Geometric mean (95% CI) antibody titres measured by glycoprotein-B enzyme-linked immunoassay
 (A) Seronegative recipients. (B) Seropositive recipients. Tx indicates the geometric mean titres found at the time of transplantation.

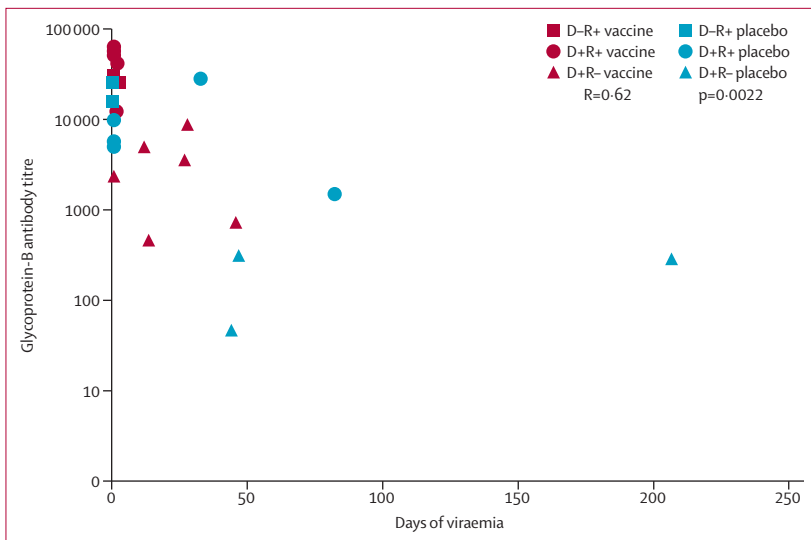


Figure 3: Inverse correlation of titre of antibodies against glycoprotein B present at the time of transplantation with duration of viraemia after transplantation
 Only patients with viraemia at any time were selected for this analysis. D=cytomegalovirus seronegative donor. R=cytomegalovirus seronegative recipient. D+=cytomegalovirus seropositive donor. R+=cytomegalovirus seropositive recipient.

Role of the funding source

See [Online](#) for webappendix

The sponsors of the study and the funding source had no role in the study design, data collection, data analysis, data interpretation, writing of the manuscript, or in the decision to submit to publication. PG, CS, VE, RM had full access to all the data. All authors reviewed the report and had final responsibility for the decision to submit for publication.

Results

No major imbalances in demographic or clinical features were noted between seropositive or seronegative patients randomly assigned to vaccine or placebo (table 1). Figure 1

shows the trial profile at Dec 31, 2009. All 140 randomised patients were evaluable for the two co-primary endpoints of safety and immunogenicity. Of the 67 patients randomly assigned to vaccine, all 67 (100%) had not proceeded to transplantation or death before the first schedule dose, 66 (98.5%) had not done so before the second dose, and 40 (59.7%) had not done so before the third dose. For the 73 patients randomly assigned to placebo, these figures were 73 (100.0%) before the first scheduled dose, 72 (98.6%) before the second dose, and 55 (75.3%) before the third dose; these numbers were the denominators in the modified missing-equals-failure analysis. 34 patients were still awaiting transplantation as of Dec 31, 2009. 78 patients proceeded to transplantation, all of whom were evaluable, with a median (range) of 95 (15278) days of follow-up.

The geometric mean titre of glycoprotein-B antibodies (figure 2) was significantly increased 1 month after the second dose (day 56) in those initially seronegative (geometric mean titre of 12537 in vaccine recipients vs 86 in placebo recipients; $p < 0.0001$) or initially seropositive (118395 vs 24682; $p < 0.0001$). The geometric mean titre of neutralising antibodies was not significantly increased at day 56 in seronegative patients ($p = 0.10$), but was significantly increased in the seropositive patients ($p = 0.0037$; webappendix p 1), in whom the neutralising antibody titres correlated with glycoprotein-B antibody titres (webappendix p 2).

Of the 67, 66, and 40 patients from the vaccine group who had not been transplanted by the time of vaccine administration, eight (12%), 18 (27%), and 20 (50%) did not complete a diary card for the first, second, and third doses, respectively. For the 73, 72, and 55 evaluable patients in the placebo group, these figures were ten (14%), 17 (24%), and 23 (42%). Injection site pain was significantly increased after dose 1 (38 of 67 [57%] patients given vaccine versus 19 of 73 [26%] patients given placebo; $p = 0.0002$

	Viraemia >200 genomes per mL	Number treated	Median days of follow-up (range)	Median number of samples (range)	Days PCR positive/total person-days of follow-up (%)*	Days treated/total person-days of follow-up (%)*
Seronegative donor, seronegative recipient (n=22)						
Placebo (n=10)	0	0	96 (51–115)	13 (6–25)	0/915 (0%)	0/915 (0%)
Vaccine (n=12)	0	0	105 (15–138)	15 (3–26)	0/1204 (0%)	0/1204 (0%)
p value	1.00	1.00
Seronegative donor, seropositive recipient (n=18)						
Placebo (n=7)	2	0	95 (65–157)	20 (8–25)	2/696 (<1%)	0/696 (0%)
Vaccine (n=11)	4	0	93 (89–176)	20 (4–26)	21/1209 (2%)	0/1209 (0%)
p value	1.00	0.59
Seropositive donor, seropositive recipient (n=22)						
Placebo (n=15)	6	3	95 (45–173)	17 (8–26)	119/1489 (8%)	135/1489 (9%)
Vaccine (n=7)	4	0	93 (91–228)	14 (7–21)	6/803 (<1%)	0/803 (0%)
p value	0.51	0.22
Seropositive donor, seronegative recipient (n=16)						
Placebo (n=5)	5	4	91 (20–278)	19 (5–62)	339/599 (57%)	415/599 (70%)
Vaccine (n=11)	6	5	94 (90–122)	19 (10–24)	128/1069 (12%)	142/1069 (13%)
p value	0.0480	0.0287
N=78	27	12

*Total number of person-days during which any participant had viraemia higher than 200 genomes per mL or received treatment divided by total number of days of follow-up for all participants who underwent a transplantation. The proportion of days of post-transplantation follow-up spent with viraemia (or receiving treatment) was calculated for each individual. These values were then compared between vaccine and placebo with a Mann-Whitney U test. Comparison of proportion of days of viraemia in (all) vaccine versus (all) placebo p=0.99.

Table 2: Cytomegalovirus viraemia and treatment in subgroups of transplant patients with defined donor and recipient serostatus

with a risk difference of 31% (95% CI 15.1–46.2). Similar results were seen after dose 2 (43 of 66 [65%] versus 23 of 72 [31%]; $p < 0.0001$) and dose 3 (31 of 40 [78%] versus 28 of 55 [51%] $p = 0.0083$). This trend remained in a missing-equals-excluded analysis. The severity of the pain was graded from mild to moderate in most cases, did not increase with the number of doses, lasted for a similar time in patients given vaccine or placebo, and was not a reason for requested withdrawal from the study.

When considering the other solicited events after administration of the first dose, no evidence was shown of increased muscle pain (30 [45%] of 67 events in vaccine group vs 23 [32%] of 73 in placebo group; $p = 0.15$), headaches (21 [31%] of 67 vs 27 [37%] of 73; $p = 0.60$), swelling (16 [24%] of 67 vs 16 [22%] of 73; $p = 0.94$), raised temperature (12 [18%] of 67 vs 12 [16%] of 73 $p = 0.99$) or redness (19 [28%] of 67 vs 20 [27%] of 73; $p = 1.00$). The findings of 277 available diary cards are summarised in the webappendix p 3, with each individual contributing up to three observations. No individuals completed diary cards after progressing to transplantation or after withdrawing from the study. Individuals could only have a maximum of one of each adverse event per injection, but could have more than one different type of adverse event and could experience the same adverse event at subsequent injection visits. 47 patients (20 given vaccine, 27 placebo) experienced serious adverse events (webappendix pp 4,5), none of which were assessed by the principal investigator as related to vaccination. The

results also summarise the unsolicited adverse events reported across all three timepoints. Overall, 108 patients (59 given vaccine, 49 placebo) reported 162 adverse events (100 in vaccine group, 62 in placebo group). Of these, 19 adverse events (15 in vaccine group, 4 in placebo group) were assessed to be related to vaccination.

After transplantation, 27 of 78 patients developed viraemia whose duration correlated inversely with the geometric mean titre of glycoprotein-B antibodies (figure 3). Of the five vaccinees who necessitated treatment (figure 3), only one had received all three doses of vaccine. The patients were well-matched for duration of follow-up and number of samples collected (table 2). Vaccinees in the subgroup of donor seropositive and recipient seronegative had a lower proportion of days on which samples were PCR positive (12% of total patient follow-up time post-transplantation versus 57%; $p = 0.0480$) and days on which treatment was given (13% vs 69%, $p = 0.0287$, table 2). The median peak viral load was 6310 genomes per mL in recipients of placebo and 562 genomes per mL in recipients of vaccine ($p = 0.34$); one placebo recipient was excluded from this calculation because she died from a surgical complication before reaching a peak viral load. Comparison of proportion of days of treatment in (all) vaccine versus (all) placebo $p = 0.31$. The magnitude of this effect is shown in figure 4. The proportion of days of post-transplantation follow-up spent with viraemia (or receiving treatment) was calculated for each individual and these values were then compared between vaccine and placebo

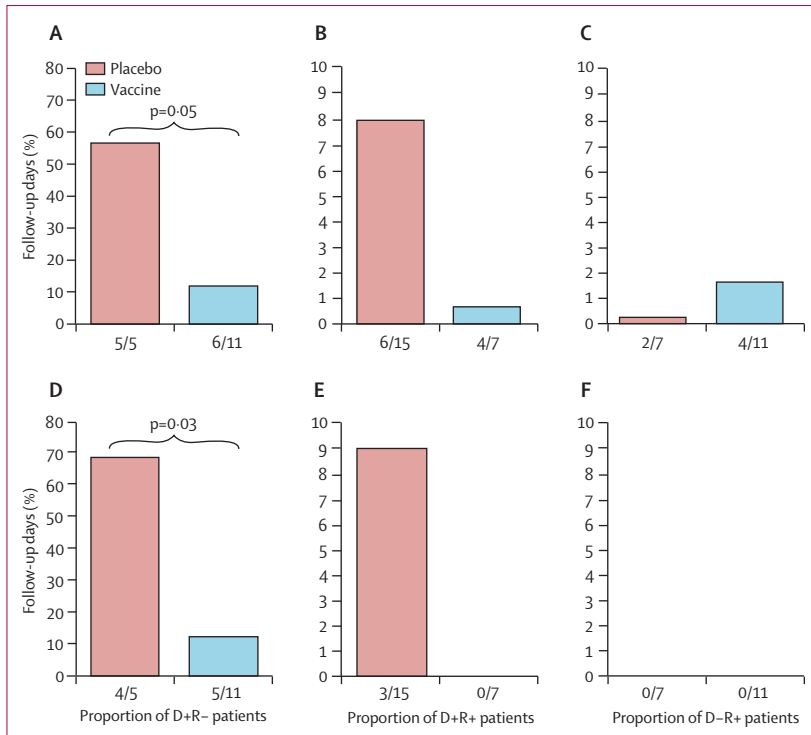


Figure 4: Proportion of days that patients in the three subgroups at risk of CMV infection spent with viraemia or received antiviral treatment

A, B, and C show the duration of viraemia. D, E, and F show the duration of antiretroviral therapy. D+R-, D+R+, and D-R+ are three groups at risk of primary infection, reinfection, and reactivation, respectively. The numbers below each column indicate the number of patients with viraemia (or treatment) divided by the number in the subgroup. Note the different values on the Y-axes of panels A and D compared with panels B, C, E, and F. D- = cytomegalovirus seronegative donor. R- = cytomegalovirus seronegative recipient. D+ = cytomegalovirus seropositive donor. R+ = cytomegalovirus seropositive recipient.

Panel: Research in context

Systematic review

We searched PubMed with the search terms “cytomegalovirus”, “vaccine”, and “placebo” for randomised controlled trials and clinical trials published in English up to March 17, 2011. Only two double-blind randomised placebo-controlled phase 2 published trials^{27,28} have attempted to control cytomegalovirus. In 1994, a study²⁷ of the live attenuated Towne vaccine in seronegative allograft recipients reported significantly reduced severity of end-organ disease related to the virus without significantly reduced incidence of virus infection.²⁷ In 2009, a glycoprotein-B/MF59 vaccine was reported to provide 50% protection to seronegative women of childbearing age against acquiring primary cytomegalovirus infection.²⁸ We used the same glycoprotein-B/MF59 vaccine and the same schedule in seronegative and seropositive transplant patients and report in this paper reduced viraemia and a correlate of immune control.

Interpretation

One vaccine could be sufficient to protect both major populations at risk of cytomegalovirus disease, which implicates glycoprotein-B as an important component of any vaccine for this virus.

groups with a Mann-Whitney *U* test. Four patients (all placebo) had a second discrete episode of viraemia, which responded to pre-emptive therapy. Only one patient (placebo recipient) developed cytomegalovirus end-organ

disease. The frequency of CD4 T-cells responsive to cytomegalovirus lysate was not increased in vaccinees at the time of transplantation (webappendix p 6).

Discussion

The antibody titre produced against the glycoprotein-B protein contained in the vaccine was significantly increased 1 month after the second injection in patients given the vaccine compared with those given placebo, both in patients who were immunologically naive to cytomegalovirus and in those with naturally acquired immunity. The antibody titres were still significantly high when the subset of 78 patients proceeded to transplantation, and correlated inversely with the duration of viraemia. Seronegative recipients with seropositive donors had a reduced duration of viraemia when given the vaccine, which translated into a reduced number of days on which pre-emptive therapy with ganciclovir or valganciclovir was given. Although the number of patients in the highest-risk donor seropositive, recipient seronegative group was small and the differences not significant, the major effect of the vaccine seemed to be on new infections acquired from the donor. Thus, we propose that antibodies induced by a cytomegalovirus vaccine might be able to bind the virus in the donated organ, thereby preventing transmission to the recipient that is sufficient to cause detectable viraemia after transplantation.

We plan to design a future randomised controlled trial to test this possibility formally. It remains to be determined whether such antibodies bind and neutralise cytomegalovirus virions released from the donated organ or whether the antibodies mediate antibody-dependent cellular cytotoxicity on virus-infected cells contained within the donated allograft. We are currently testing sera to differentiate between these two possibilities.

This phase 2 proof-of-concept study expands the limited amount of published information about cytomegalovirus vaccines (panel). We minimised sources of bias by randomising patients to receive vaccine or placebo. Nevertheless, the number of patients in each subset is small, so the encouraging results we report should be confirmed in definitive phase 3 studies. Since viraemia is a prerequisite for development of cytomegalovirus disease,^{14,26} the prevention of viraemia should translate into the prevention of end-organ disease. This theory cannot be formally studied in patients receiving pre-emptive treatment because of the low incidence of end-organ disease, but could be assessed in patients managed with antiviral prophylaxis, some of whom remain at risk of disease once prophylaxis is stopped.^{13,29} A randomised controlled trial of prophylaxis with immunoglobulin³⁰ lends support to the notion that antibodies can reduce cytomegalovirus end-organ disease. The boosting of antibody responses in natural seropositive people is also interesting and could be assessed in other populations such as women

of childbearing age who are at risk from cytomegalovirus reinfection.

The beneficial effects of the cytomegalovirus glycoprotein-B/MF59 vaccine were obtained with an acceptable safety profile. The only adverse event significantly increased in recipients of vaccine was pain at the site of injection, as was seen previously in healthy volunteers.^{20,21} We conclude therefore that vaccines containing cytomegalovirus glycoprotein-B should be assessed further in transplanted patients.

Contributors

All authors contributed to the study design drafted by PDG. AS, MH, AD, GJ, DCW, JO'B, DT, DP, PS, ER, NO, RK, SL, SA, TH, and AKB contributed to the clinical implementation of the study and supervision of the patients. CS designed and did the statistical analysis, and verified its accuracy. PDG and VCE contributed to planning of protocol-stated analyses. EM, MO, CEA, TH, SA, Spr, SL, and RM did laboratory analyses. SPi, ML, and EW supervised provision of vaccine and placebo. PDG, CS, VCE, RSBM, MH, DCW, and AKB helped draft this report or critically revise the draft. All authors reviewed and approved the final version of the report.

Conflicts of interest

PDG has been a member of advisory boards for Viropharma, Chimerix, Astellas, and Boehringer-Ingelheim. DCW has received consulting fees from Amgen and lecture payments from Amgen and Shire. PS has been a member of advisory boards from Wyeth, Novartis, Roche, and Astellas. VCE has acted as a member of the advisory board, received consulting fees and lecture fees from Roche and Viropharma. SPi is an employee of Sanofi Pasteur. All other authors declare that they have no conflicts of interest.

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