Viraemia, immunogenicity, and survival outcomes of cytomegalovirus chimeric epitope vaccine supplemented with PF03512676 (CMVPepVax) in allogeneic haemopoietic stem-cell transplantation: randomised phase 1b trial


Summary

Background Patients seropositive for cytomegalovirus (CMV) and undergoing allogeneic haemopoietic stem-cell transplantation (HCT) are at risk for CMV reactivation. Stimulating viral immunity by vaccination might achieve CMV viraemia control without the need for antiviral agents. CMVPepVax is a chimeric peptide composed of a cytotoxic CD8 T-cell epitope from CMV pp65 and a tetanus T-helper epitope. It is formulated with the adjuvant PF03512676, a Toll-like receptor 9 agonist, which augments cellular immunity. We aimed to assess safety, immunogenicity, and possible clinical benefit of the CMVPepVax vaccine in patients undergoing HCT.

Methods We did a randomised, open-label, phase 1b trial at one transplant centre in the USA. Eligible patients were CMV-seropositive, positive for HLA-A*0201, aged 18–75 years, and undergoing HCT from a matched-related or matched-unrelated donor. Patients were reassessed for eligibility on day 28 after HCT. We randomly allocated patients to either the CMVPepVax vaccine or observation, in blocks stratified by CMV donor serostatus. CMVPepVax was administered subcutaneously on days 28 and 56. The primary outcome was safety, which consisted of secondary graft failure, grade III–IV acute GVHD, non-relapse mortality by day 100, serious adverse events related to the vaccine (judged by the data and safety monitoring committee [DSMC]) grade 3–4 adverse events related to the vaccine (judged by the DSMC) within 2 weeks of vaccination, and development of double-strand (ds) DNA autoantibodies. Statistical analyses included all randomised patients and were done per-protocol. This study is registered with ClinicalTrials.gov, number NCT01588015. This trial is closed to accrual and the final analysis is presented in this report.

Findings Between Oct 31, 2012, and Nov 5, 2014, 36 eligible patients were allocated to either CMVPepVax (n=18) or observation (n=18), with no adverse effect on HCT (no secondary graft failures in either group) or cases of acute GVHD (seven patients assigned vaccine and six under observation had acute GVHD of grade 2 or less), and no unexpected adverse events. Compared with observation, better relapse-free survival was recorded in patients allocated the vaccine (seven vs one; hazard ratio [HR] 0·12, 95% CI 0·01–0·94; p=0·015). No patients had non-relapse mortality by day 100. One serious adverse event (grade 1 fever) was attributed to CMVPepVax but resolved within 48 h. Four patients assigned the vaccine had a serious adverse event, which was unrelated to the vaccine (grade 3 thrombocytopenia, grade 3 device-related infection, grade 2 nausea, and grade 1 fever), compared with nine patients under observation (grade 4 maculopapular rash, grade 3 nausea, grade 3 infection, grade 3 thrombotic thrombocytopenic purpura, grade 2 nausea, grade 2 generalised muscle weakness, grade 2 infection, grade 1 fever, and grade 1 fatigue; p=0·16). 54 grade 3–4 adverse events were reported in patients assigned the vaccine compared with 91 in patients who were under observation (p=0·2). No patients had grade III–IV acute GVHD or developed dsDNA autoantibodies.

Interpretation The results show safety and immunogenicity of the CMVPepVax vaccine. The prospect of substantial clinical benefits warrant testing in a phase 2 trial.

Funding National Cancer Institute.

Introduction

Allogeneic haemopoietic stem-cell transplantation (HCT) has curative properties for many haematological disorders. Early after HCT, both innate and adaptive immunity are impaired because of immunosuppression associated with the procedure. As a result, patients undergoing HCT are highly susceptible to opportunistic infections. Despite pre-emptive antiviral treatment, cytomegalovirus (CMV) remains the leading infectious complication in patients who have undergone HCT. CMV reactivation mainly happens within the first 100 days after HCT and in more than a third of patients seropositive for CMV, the population at highest risk for CMV reactivation. Because of early CMV reactivation after HCT, and enhanced risk of severe end-organ disease, individuals who are seropositive for CMV—either the donor or the recipient—have greater non-relapse mortality and poorer overall survival. Current antiviral treatment limits...
viraemia effectively; however, its use is associated with toxic effects, which besides adding to the cost of HCT creates delays in immune reconstitution, increases fungal and bacterial infections, augments breakthrough gastrointestinal CMV disease, and amplifies the risk of fungal and bacterial infections, augments breakthrough viraemia requiring antiviral therapy, and pp65 and glycoprotein B immune responses were not increased significantly, using the protocol-specific statistical analysis. An international, randomised, double-blind, placebo-controlled, phase 3 study of ASP0113 is ongoing and aims to enrol 500 CMV-seropositive patients (NCT01877655). The main objective is to ascertain whether overall mortality 1 year after allograft is reduced in patients given ASP0113.

**Added value of this study**

In patients undergoing HCT who were CMV-seropositive and positive for HLA-A*0201, the CMVPepVax vaccine achieved control of CMV viraemia without the need for costly and potentially toxic antiviral drugs. Our findings add value to existing evidence showing that immunosuppressed patients undergoing HCT might respond to vaccination early after HCT, when they are at enhanced risk for CMV reactivation. To our knowledge, our data with CMVPepVax provide proof of concept for a CMV vaccine in the HCT setting to show vaccine-induced increase in pp65-specific CD8 T cells, protection from CMV reactivation, and reduced use of antivirals. The unexpected clinical outcomes of reduced relapse and increased survival with CMVPepVax are also, to our knowledge, proof of concept that a vaccine stimulating both innate and adaptive arms of the immune response can increase relapse-free survival by achieving clinical benefits for a wide range of major complications after HCT.

**Implications of all the available evidence**

Our data confirm previous findings suggesting that humoral immunity is not needed for CMV viraemia control after HCT. Thus, addition of glycoprotein B—as in the ASP0113 vaccine—is probably unnecessary in the HCT setting, although previous CMV vaccines used in the setting of solid-organ transplantation have shown a correlation between humoral immunity (anti-glycoprotein B) and suppression of CMV viraemia. Developing a protective CMV vaccine, which might also reduce relapse and increase survival, will affect HCT clinical practice favourably and increase the curative potential of HCT. Clinical outcomes of CMVPepVax have led to initiation of a phase 2, multicentre, placebo-controlled trial that, combined with findings of the ongoing phase 3 ASP0113 clinical trial, will provide definitive characterisation of the properties of these promising candidate vaccines for the HCT setting (NCT02396134).
viraemia was extended and CMV viraemia levels were lower in vaccinated patients.13,14 The unmet need for a CMV vaccine in the HCT setting prompted development of CMVPepVax, an investigational CMV vaccine composed of the HLA-A*0201-restricted pp65 CD8 T-cell peptide epitope fused with the P2 peptide epitope of tetanus toxin and mixed with a Toll-like receptor 9 (TLR9) agonist, PF03512676, as an adjuvant just before administration. An acceptable safety profile and vaccine-driven expansion of pp65 T cells in healthy adults when used with PF03512676 supported further assessment in patients undergoing HCT.15 The CMVPepVax vaccine aims to stimulate a CD8 T-cell response directed towards pp65495–503, a dominant T-cell epitope of the pp65 tegument protein involved in CMV viraemia protection early after HCT.13,15 The aim of the present study was to assess the safety, immunogenicity, and clinical benefit of the CMVPepVax vaccine in patients seropositive for CMV and undergoing HCT who are at high-risk for CMV reactivation and end-organ disease.1 This trial is closed to accrual and has reached the stage of patients’ follow-up; the final analysis is presented here.

Methods
Study design and patients
We did a randomised, open-label, phase 1b trial at the City of Hope Comprehensive Cancer Center (Duarte, CA, USA). We recruited patients from among those scheduled to undergo allogeneic HCT for haematological malignant diseases. According to the study centre’s standard of care, HCT is permitted in patients with a Karnofsky performance status of 60 or higher and estimated survival of longer than 3 months. We judged patients eligible if they were CMV-seropositive, positive for HLA-A*0201, aged 18–75 years, and willing to be monitored for at least 6 months, and had either a related or unrelated donor with 8/8 or 7/8 (HLA-A, HLA-B, HLA-C, and HLA-DRB1) high-resolution HLA donor allele-matching. The protocol was amended to include 7/8 matched-related and 7/8 or 8/8 matched-unrelated donors (version 8; July 21, 2013). About two-thirds of patients undergoing HCT were CMV-seropositive, of which 34% were HLA-A*0201-positive and candidates for the current CMVPepVax vaccine. We applied further eligibility restrictions for safety and to facilitate follow-up. Additional exclusion criteria included receiving T-cell depleted HCT, aplastic anaemia, acute leukaemia not in remission, receipt of a live-attenuated vaccine within 30 days after HCT, previous treatment for CMV viraemia, congenital or acquired immune deficiencies, autoimmunode disease, HIV, hepatitis C, and active hepatitis B positivity (surface antigen negative).

The study protocol is available to view online. The study was approved by the local institutional review board and the US Food and Drug Administration (FDA; investigational new drug BB-13124) before enrolment began. All participants gave written informed consent.

Randomisation and masking
Randomisation was done on day 28 after HCT. We used a computer-generated randomisation schedule in blocks of four and stratified by CMV donor serostatus. We assigned patients in a 1:1 ratio to either the CMVPepVax vaccine or observation. The registrar concealed assignments until consent and eligibility at day 28 were established.

Procedures
We permitted use of myeloablative, reduced intensity, and non-myeloablative conditioning regimens before HCT. Selection of the conditioning regimen adhered to the study centre’s standard treatment and practice guidelines. Fully ablative regimens included fractionated total body irradiation (12 Gy) plus etoposide (60 mg/kg) or cyclophosphamide (100 mg/kg). Reduced intensity regimens were nearly ablative because they included melphalan (100–140 mg/m²) in combination with fludarabine (125 mg/m²) or clofarabine (30–40 mg/m²), resulting in greater than 90% donor T-cell engraftment by day 30 in most patients.1 The adjuvant rituximab in patients with B-cell lymphoma.

On day 28 after HCT, we reassessed each enrolled patient for eligibility. We excluded individuals from the study if they had failed to engraft (defined as the first of 3 consecutive days when the peripheral blood absolute neutrophil count is ≥5×10⁸ cells per L), had CMV reactivation (≥500 genomic viral copies [gc] per mL), had grade III–IV acute GVHD (according to the Keystone Consensus grading system), received a dose of steroids greater than 1 mg/kg per day within 7 days of immunisation, or had any ongoing non-haematological toxic effect of grade 3 or 4 (according to the Common Terminology Criteria for Adverse Events [CTCAE] version 4.03).

The peptide portion of the CMVPepVax vaccine (namely, NSC-721434) is produced by the National Cancer Institute-funded Rapid Access to Interventional Development programme in contract with Bachem (Torrance, CA, USA). NSC-721434 consists of the HLA-A*0201-specific pp65495–503, CD8 T-cell epitope fused with the P2 epitope of tetanus toxin (appendix p 2).13,16 The adjuvant, PF03512676, is a synthetic single-stranded phosphorothioate DNA-containing CpG motifs (24 nucleotides in length) that was supplied by Pfizer (New York, NY, USA) and is classified as an investigational agent (appendix p 2). The CMVPepVax vaccine formulation is comprised of 2.5 mg of the NSC-721434 peptide vaccine solution and 1.08 mg of the PF03512676 adjuvant, in a final 1 mL injection volume (appendix p 2). In the CMVPepVax healthy volunteer trial,19 four doses of the vaccine were administered 3 weeks apart to assess safety of multiple injections. Because we did not know whether use of an agent augmenting cellular immunity (eg, PF03512676) could have affected the development or severity of acute GVHD, we decided to administer only two doses of the...
We adhered to the study centre’s standard of care for administration of immunosuppressive therapy; tapering typically started on day 100 after HCT, in the absence of GVHD. Thus, patients in both study groups were under full immunosuppressive coverage during the first 100 days after HCT. We did not administer T-cell depleting agents (eg, antithymocyte globulin) or an ex-vivo T-cell-depleted graft. We gave supportive care, including prophylactic antibiotics, antifungal therapy, total parental nutrition, haemopoietic growth factors, immunoglobulin replacement, and treatment of mucositis and neutropenic fever, in accordance with institutional standard practice guidelines. GVHD prophylaxis consisted of tacrolimus and sirolimus. All patients received standard-dose aciclovir for treatment of herpes simplex virus and varicella zoster virus. We allowed pre-emptive management of CMV infection, but we prohibited planned use of prophylactic CMV antivirals or CMV immunoglobulin.

We monitored all patients for GVHD and adverse events as necessary and not less than every 2 weeks from day 28 until day 100 after HCT. We graded adverse events with CTCAE version 4.03. Afterwards, we monitored GVHD as necessary or every month until 6 months, and subsequently as per the study centre’s standard of care.

We assessed CMV viraemia by quantitative PCR methods with probes designed to detect the UL83 and UL55 genes of the CMV genome. Further details are proprietary to either the study centre or Focus Diagnostics (Cypress, CA, USA), but meet the requirements of the FDA Clinical Laboratory Improvement Amendments (CLIA) for clinical diagnostic laboratories. The current assay from Focus Diagnostics (Simplexa CMV Kit) is FDA-approved. Quantitative results were reported of 500–500 000 gc/mL (1 gc/mL is equivalent to 2·5 IU/mL). This test was developed and its performance characteristics determined by the study centre’s clinical molecular diagnostic laboratory (certified under CLIA, 1988). We did monitoring for CMV viraemia by quantitative PCR twice a week from day 21 to day 100 after HCT, and thereafter we implemented rigorous patient-based and risk-adapted monitoring, according to the study centre’s standard of care. According to the study centre’s standard of care, patients undergoing HCT with a matched-related or matched-unrelated donor should receive pre-emptive anti-CMV therapy (ganciclovir, valganciclovir, or foscarnet) when the CMV PCR value rises to 1500 gc/mL or higher. Pre-emptive therapy was recommended for CMV PCR values lower than 1500 gc/mL only when patients were judged at high risk for CMV disease, such as when receiving high-dose steroids (appendix p 1).

Occurrence of CMV disease, and number of CMV-specific antiviral treatment days were recorded up to 6 months. We monitored haematological relapses and deaths until May 31, 2015. As required by the FDA, since the CpG-DNA portion of the CMVPepVax vaccine has been associated with autoimmunity, in patients allocated the vaccine, we measured amounts in serum of double-strand (ds) DNA IgG autoantibody with Wampole DS DNA ELISA II (Alere, Orlando, FL, USA) at days 28, 56, 100, and 180 after HCT.

We monitored CMV-specific immunogenicity in peripheral blood mononuclear cells (PBMCs) of all enrolled recipients every 2 weeks from day 28 until day 100 after HCT, then at days 130, 160, and 180, by measuring amounts of CD8 T cells binding to MHC class I pp65495–503 and HIV gag77–85 pentamers (ProImmune, Oxford, UK). In patients allocated the vaccine, we measured amounts in serum of double-strand (ds) DNA IgG autoantibody with Wampole DS DNA ELISA II (Alere, Orlando, FL, USA) at days 28, 56, 100, and 180 after HCT. We monitored CMV-specific immunogenicity in peripheral blood mononuclear cells (PBMCs) of all enrolled recipients every 2 weeks from day 28 until day 100 after HCT, then at days 130, 160, and 180, by measuring amounts of CD8 T cells binding to MHC class I pp65495–503 and HIV gag77–85 pentamers (ProImmune, Oxford, UK). In patients allocated the vaccine, we measured amounts in serum of double-strand (ds) DNA IgG autoantibody with Wampole DS DNA ELISA II (Alere, Orlando, FL, USA) at days 28, 56, 100, and 180 after HCT.

A data and safety monitoring committee (DSMC) at the study centre reviewed and monitored toxic effects and accrual data from this trial. The DSMC included clinical specialists at the study centre with experience in oncology, who had no direct relationship with the study. The DSMC reviewed up-to-date participant accrual, a summary of all adverse events captured via routine and expedited reporting, a summary of deviations, any response information, monitoring reports, and summary comments provided by the study team. Study audit reports were provided to the DSMC by the study centre’s Office of Clinical Trials Auditing and Monitoring.

**Outcomes**

The primary outcome was safety of the CMVPepVax vaccine; key safety endpoints were secondary graft failure, grade III–IV acute GVHD, non-relapse mortality by day 100, serious adverse events related to the vaccine
(judged by the DSMC), grade 3–4 adverse events related to the vaccine (judged by the DSMC) within 2 weeks of vaccination, and development of dsDNA autoantibodies. We monitored the safety of all patients on an ongoing basis up to day 180 after HCT.

A secondary objective was to assess the CD8 T-cell response to pp65\textsubscript{495-503} between study groups. Additional endpoints included clinical and laboratory evaluation of CMV reactivation and disease, acute and chronic GVHD, relapse-free survival, non-relapse mortality, and the number of patients relapsing after HCT.

**Statistical analysis**

We included all randomised patients in the analysis of clinical outcomes and did analyses as randomised, whereas the analysis of T-cell response focused on patients who did not reactivate CMV. We based the safety assessment on protocol-specified monitoring rules and the summary of adverse events. We compared differences between study groups in serious adverse events and adverse events by two-sided rank-sum test, and Fisher’s exact test. We estimated the cumulative incidence of CMV viraemia by the product-limit method. We used Cox proportional hazards models to estimate hazard ratios, with significance based on log-rank tests, and we checked for qualitative agreement with likelihood ratio tests. We used similar methods to compare the relative hazard of other events across study groups. We censored patients at relapse with respect to assessment of CMV reactivation and non-relapse mortality.

In the main assessment of immunogenicity, we omitted patients with CMV reactivation to distinguish the effect of vaccination from that of exposure to replicating CMV. In a protocol-specified analysis, we compared levels of CD8 T cells specific for CMV pp65\textsubscript{495-503} between study groups by Wilcoxon rank-sum test, using integrated post-vaccination CMV pp65\textsubscript{495-503}-specific CD8 T-cell levels to day 100 after HCT, as a numerical outcome. We did this analysis on a logarithmic scale to represent individual responses relative to baseline levels (day 28 after HCT), which had highly variable and skewed distributions, characteristic of the wide variability of immune reconstitution timing after HCT.\textsuperscript{22}

We planned for the trial to have 90% power for the rank-sum test to detect a vaccine effect that would place about 90% of vaccine recipients with CMV pp65\textsubscript{495-503}-specific CD8 T-cell concentrations characteristic of the upper 50% of patients under observation, a shift such that four equally likely intervals in the observation arm would be occupied with probabilities of 4%, 8%, 17%, and 71% (proportional odds model). This plan yields 91% power at a one-sided 0.05 level of significance, calculated with StatXact/Cytel Studio version 7.0.0. As a more probative analysis, we used generalised estimating equation (GEE) methods to model the marginal means, on a log scale, as functions of vaccination, baseline CMV pp65\textsubscript{495-503}-specific CD8 T-cell concentrations, time after HCT, and donor CMV serostatus. We did analyses with R version 3.1.2 with the survival (version 2.37-7) and GEE (version 4.13-18) packages (available from the Comprehensive R Archive Network).

This trial is registered at ClinicalTrials.gov, number NCT01588015.

**Role of the funding source**

The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

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**Figure 1: Trial profile**

CMV=cytomegalovirus. HCT=haemopoietic cell transplantation. *Before protocol amendment to allow 7/8 match. †Investigator judged ineligible. ‡One research nurse was responsible for all HCT protocols and could not enrol all potential participants.
Articles

Results
Between Sept 19, 2012, and Sept 29, 2014, 130 patients were screened for eligibility and, of these, 68 were deemed ineligible (figure 1). 46 eligible patients scheduled for HCT were enrolled into the trial. CMV did not reactivate in any patient by day 28 after HCT, at which time all individuals were reassessed for eligibility. Six patients did not meet criteria for randomisation (one patient received steroids >1 mg/kg per day, two had grade 3 acute GVHD, and three had ongoing grade 3–4 adverse events). Furthermore, three patients withdrew and one individual died early after HCT before day 28 (figure 1). Therefore, between Oct 31, 2012, and Nov 5, 2014, 36 eligible patients were randomised to either the CMVPepVax vaccine (n=18) or observation (n=18). Four (22%) patients assigned the vaccine did not receive the second CMVPepVax injection: two became ineligible according to the protocol, because they needed CMV antiviral treatment; one patient declined the second vaccine injection; and one patient had ongoing grade 3 toxic effects related solely to HCT, and the treating clinician decided to withhold the second vaccine injection (figure 1). Four (22%) patients under observation had CMV reactivation before day 56 and would have been ineligible for vaccine injection. Follow-up was curtailed for three patients under observation because of relapse at days 65, 77, and 97. Furthermore, one patient under observation relocated and withdrew consent at day 130 for further blood draws, but follow-up for relapse-free survival continued (figure 1). These patients were all included in the primary analysis.

Baseline characteristics were closely balanced between the two groups (table 1). All patients received peripheral blood stem-cell grafts. 23 (64%) patients had reduced-intensity conditioning and the remainder had a fully ablative regimen. 11 (61%) patients assigned the vaccine had a matched-unrelated donor compared with six (33%) individuals under observation. Despite this difference, Karnofsky performance score at randomisation and the overall Disease Risk Index—the strongest determinant of survival after HCT—was balanced between study groups (table 1).

Immunosuppressive treatment was similar between study groups. The median prednisone daily dose over the first 56 days (data gathered as specified in the protocol) was 382.5 mg (IQR 0–776.2) for patients under observation and 535 mg (I92.5–820) for those assigned the vaccine. Respectively, median tacrolimus daily doses were 62.5 mg (IQR 57.25–75.6) and 93.5 mg (64–115.2), and median sirolimus daily doses were 95 mg (IQR 60–148.2) and 164 mg (116–228.8). Moreover, at day 100 and day 180, the median prednisone daily dose was 0 mg (IQR 0–10) and 3.75 mg (0–10), respectively, in patients under observation, and 2.5 mg (IQR 0–10) and 0 mg (0–8.75), respectively, in those assigned the vaccine. A short course of methotrexate was given to patients undergoing HCT from a 7/8 matched donor (either 5 mg/m² on days 1, 3, and 6 after HCT or 15 mg on day 1 after HCT and 10 mg/m² on days 3, 6, and 11 after HCT).

No patients assigned the vaccine met predefined stopping rules after the first or second injection, and none needed a dose reduction or discontinued for a drug-related toxic effect. No vaccine-related deaths were recorded. Follow-up for adverse events was from the first injection on day 28 to day 100 after HCT, with onset of recorded adverse events ranging from day 23 to day 103. The median duration of follow-up for adverse events was 58 days (IQR 44–74). One patient assigned the vaccine had a serious adverse event (defined in the online protocol) that was attributed to the second CMVPepVax injection (grade 1 fever on the day of vaccination) and needed hospital admission; fever resolved within 48 h. We recorded serious adverse events in four patients assigned the vaccine (grade 3 thrombocytopenia, grade 3 device-related infection, grade 2 nausea, and grade 1 device-related infection). The median duration of follow-up for adverse events was 58 days (IQR 44–74). One patient assigned the vaccine had a serious adverse event (defined in the online protocol) that was attributed to the second CMVPepVax injection (grade 1 fever on the day of vaccination) and needed hospital admission; fever resolved within 48 h. We recorded serious adverse events in four patients assigned the vaccine (grade 3 thrombocytopenia, grade 3 device-related infection, grade 2 nausea, and grade 1

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<td>12 (67%)</td>
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Data are number of patients (%) or median (range). CMV=cytomegalovirus. HCT=haemopoietic cell transplantation.

Table 1: Baseline characteristics
fever), which were unrelated to the vaccine, and nine patients under observation (grade 4 maculopapular rash, grade 3 nausea, grade 3 infection, grade 3 thrombocytopenic purpura, grade 2 nausea, grade 2 generalised muscle weakness, grade 2 infection, grade 1 fever, and grade 1 fatigue). The number of serious adverse events, and adverse events of grades 3 and 4, did not differ between study groups (table 2; appendix pp 3, 4). Local and systemic skin reactions were negligible in patients assigned the vaccine in our trial (data not shown). No patients assigned the vaccine developed dsDNA autoantibodies after the injections, up to 6 months after HCT (appendix p 5). There were no secondary graft failures in the study population.

The incidence of acute GVHD did not differ between study groups (table 2). No patients had grade III–IV acute GVHD after CMVPePvax vaccination. Chronic GVHD had similar incidence in both study groups and developed in 15 patients assigned the vaccine and 13 under observation.

The median duration of follow-up for relapse-free survival was 461 days (IQR 336–644). By May 31, 2015, all 18 patients assigned vaccine were alive, including one who developed relapse of their underlying disease about 17 months (531 days) after HCT. By contrast, five patients under observation had a relapse of haematological malignant disease and seven people died (five due to relapse; HR 0.12, 95% CI 0.01–0.94; p=0.015, two-sided likelihood ratio test; figure 2). No study patients suffered non-relapse mortality by day 100; however, two patients under observation died from non-relapse mortality after day 100, one from liver failure caused by steroid-refractory chronic GVHD and the other from complications of pneumonia caused by a bacterial lung infection.

Figure 3 shows the change in concentration of pp65_{495–503}-specific CD8 T cells from day 28 to day 180 after HCT in patients who did not subsequently have CMV reactivation (n=17 vaccine, n=11 observation). As expected, there was wide variability in T-cell levels between study groups (appendix p 6). Nonetheless, the vaccine was associated with a 3·5 times (95% CI 1·6–7·9) increase in the concentration of pp65_{495–503}-specific CD8 T cells from day 28 to day 100 after HCT (p=0.0018), compared with a 1·4 times (95% CI 0·6–3·3) rise in pp65_{495–503}-specific CD8 T cells with observation in the same period (p=0.27). The protocol-specified rank-sum test indicated a 2·0 times (95% CI 1·04–3·51) greater rise in concentrations of pp65_{495–503}-specific CD8 T cells with the vaccine, averaged to day 100, than with observation (p=0.025), as did direct comparison of study groups in GEE models (2·5 times greater increase from baseline to plateau beyond day 100; p=0.046). Donor CMV serostatus did not significantly affect pp65_{495–503}-specific CD8 T-cell concentrations. In patients seropositive for CMV, concentrations of pp65_{495–503}-specific CD8 T cells were 23% (SE 42) higher than in individuals who were seronegative for CMV (p=0.57). Highly variable pp65_{495–503}-specific T-cell profiles were noted among patients with CMV viraemia, possibly attributable to the different times or levels of CMV reactivation after HCT and the length or type of antivirals administered. After resolution of CMV viraemia, pp65_{495–503}-specific T-cell concentrations generally increased in patients who previously had viraemia, but they did not exceed amounts recorded in patients assigned vaccine who did not have CMV reactivation. The donor T-cell median around day 30 after HCT was 97.9% (range 86.4–100) in patients under observation and 97.8% (73.8–100) in those assigned the vaccine. Thus, the effect of residual host T cells was negligible.

### Table 2: Selected safety outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Vaccine (n=18)</th>
<th>Observation (n=18)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with serious adverse events</td>
<td>4 (22%)</td>
<td>9 (50%)</td>
<td>0.16*</td>
</tr>
<tr>
<td>Patients with serious adverse events related to vaccine</td>
<td>1</td>
<td>NA</td>
<td>-</td>
</tr>
<tr>
<td>Grade 3–4 adverse events</td>
<td>54</td>
<td>91</td>
<td>0.21†</td>
</tr>
<tr>
<td>Patients with acute GVHD 28 days after HCT</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
<td>0.74†</td>
</tr>
<tr>
<td>Grade I</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
<td>-</td>
</tr>
<tr>
<td>Grade II</td>
<td>6 (33%)</td>
<td>5 (28%)</td>
<td>-</td>
</tr>
<tr>
<td>Grade III–IV</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Disease relapse</td>
<td>1 (6%)</td>
<td>5 (28%)</td>
<td>0.015</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>7 (39%)</td>
<td>-</td>
</tr>
<tr>
<td>CMV viraemia (≥500 gc/mL)</td>
<td>1 (6%)</td>
<td>6 (33%)</td>
<td>0.044†</td>
</tr>
<tr>
<td>CMV disease (gastrointestinal)</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
<td>0.76†</td>
</tr>
<tr>
<td>Duration of pre-emptive CMV treatment (days)§</td>
<td>15</td>
<td>263</td>
<td>0.015†</td>
</tr>
</tbody>
</table>

Data are number (%). p values are two-sided, unless otherwise stated. Patients were followed up for at least 180 days after HCT, or until May 31, 2015. CMV=cytomegalovirus. gc=genomic viral copies. GVHD=graft-versus-host disease. HCT=haemopoietic cell transplantation. NA=not applicable. *Fisher's exact test. †Rank-sum test. ‡One-sided test.

### Figure 2: Kaplan-Meier estimates of relapse-free survival

Patients were followed up to May 31, 2015. HCT=haemopoietic cell transplantation. HR=hazard ratio.
In the one patient assigned the vaccine in whom CMV reactivated, a modest increase was noted in concentration of pp65495–503-specific CD8 T cells (0·43 cells per μL) on day 42, 15 days after the first injection. Treatment with antivirals started on day 47; leukopenia (white blood cell count 1·9 × 10⁹ cells per L) and a concomitant drop in concentration of pp65495–503-specific CD8 T cells to detection limits were recorded on day 56. A substantial increase in the amount of pp65495–503-specific CD8 T cells was noted after day 100 (>2 cells per μL).

14 (39%) patients in the total study population were tested for CMV viraemia ten times or more between day 100 and day 180, with some patients being monitored every 2 weeks up to day 180. The median number of PCR assays after day 100 was 7·0 (IQR 3–10) in patients allocated the vaccine and 7·5 (4.5–11.5) for those under observation. CMV reactivated in seven enrolled patients; six had reactivation before day 100 and one had late CMV reactivation on day 146.3 CMV reactivation was significantly lower with the vaccine compared with observation. We recorded detectable viraemia (≥500 gc/mL) in one patient assigned the vaccine compared with six patients under observation (HR 0·14, 95% CI 0·02–1·1; p=0·039, two-sided log-rank; figure 4).

Reactivation started at day 47 after HCT in the patient assigned vaccine (donor was CMV-negative) and at days 35, 37, 38, 39, 59, and 146 in those under observation. In one of the six patients under observation who reactivated and who had CMV viraemia on day 37, gastrointestinal CMV disease developed, with both positive CMV histopathology and tissue culture evidence of virus at day 130 after HCT. In one patient assigned the vaccine (both donor and recipient were CMV-positive), gastrointestinal endoscopy was done because of acute gastrointestinal GVHD: Clostridium difficile was isolated and CMV histopathology was positive on day 40 after HCT. This patient did not develop CMV viraemia (below the limit of detection) before or after the biopsy and the tissue culture was negative for evidence of CMV. There was no evidence that the absence of CMV viraemia in this patient was an effect of the first CMVPepVax injection. The treating clinicians decided to provide antiviral treatment for 60 days for a presumptive diagnosis of CMV gastroenteritis; as a result, the second vaccine injection was not administered, according to the protocol.

Duration of pre-emptive antiviral administration differed significantly between study groups. The six patients under observation in whom CMV reactivated spent a total of 263 days on antiviral drugs (138 days of induction doses and 125 days of maintenance) compared with 15 days (7 days induction, 8 days maintenance) for the patient assigned the vaccine who developed CMV viraemia (p=0·039, two-sided rank-sum test; table 2). In that one patient assigned vaccine, CMV became undetectable by PCR 4 days after treatment began, and since the patient developed renal insufficiency and pancytopenia, oral valganciclovir was discontinued earlier than recommended in the study centre’s standard of care (2 weeks of induction and 2 weeks of maintenance). All other patients followed antiviral treatment regimens as per the standard of care at the study centre.

**Discussion**

The findings of this phase 1b randomised trial showed that CMVPepVax, a novel CMV peptide vaccine formulated with the TLR9 agonist adjuvant PF03512676,
was safe and well tolerated in patients seropositive for CMV. HLA-A*0201-positive, and undergoing HCT. Compared with observation, patients given the CMVPepVax vaccine constituted significantly higher levels of pp65 495–503-specific CD8 T cells during the first 100 days after HCT and had less CMV reactivation, lower antiviral use, and longer relapse-free survival. CMV reactivation causes major health complications, profound defects in immune reconstitution, and substantial morbidity in immune-compromised patients undergoing HCT, thus diminishing the full curative potential of this successful cancer treatment.\textsuperscript{19,22} This trial was designed to assess safety and clinical outcomes of patients receiving the CMVPepVax vaccine who are at enhanced risk for CMV reactivation (ie, CMV-seropositive and undergoing HCT) and, thus, are most likely to receive antiviral drugs. Since CMV reactivation is mainly controlled by CMV-specific T cells,\textsuperscript{22,24} vaccine injections were given with the intention of eliciting a protective immune response preceding CMV reactivation. Data from our group and from an analysis by the Center for International Blood and Marrow Transplant Research (CIBMTR) indicate that the median time to CMV reactivation after HCT is about 40 days (range 1–362), with 98% of reactivations occurring before day 100. Thus, our vaccine dosing schedule (day 28 and day 56 after HCT) directly targets the period of greatest risk for CMV reactivation after HCT.\textsuperscript{25} In our study, none of the 36 patients enrolled had CMV reactivation before day 28 after HCT. The timing chosen for the first injection of vaccine seems adequate based on both safety and favourable clinical outcomes, and it allows patients to have sufficient time to engraft and recover from the acute toxic effects of HCT. Moreover, in a prospective study from our group,\textsuperscript{22} timing of initiation of the measurable CMV-specific adaptive immune response in patients undergoing allogeneic HCT was strengthened on day 28 after the procedure. As a result, vaccination at an earlier time after HCT might result in low efficacy because of limited haemopoietic reconstitution.\textsuperscript{25} Based on data from CIBMTR, follow-up to day 180 is consistent for a phase Ib trial, but we recognise the possible limitation.

By contrast with healthy volunteers,\textsuperscript{3} vaccinated patients had few injection-site reactions. Although further studies will be needed to fully understand the mechanism of this difference, immunosuppression associated with the HCT procedure is likely to reduce the injection-site reaction to CMVPepVax. Cutaneous energy to Mantoux tuberculin testing has been reported in immune-suppressed individuals, including transplant recipients, and HIV-infected people.\textsuperscript{19}

Our study was the first time CMVPepVax had been used in patients undergoing HCT; thus, strict eligibility rules were applied. As a result, only a third of the target population of CMV-seropositive and HLA A*0201-type patients was enrolled, which is a limitation of this study.\textsuperscript{25} Moreover, reassessment at day 28 after HCT ensured randomisation into the trial of patients who had engrafted, did not reactivate CMV, had not had HCT-related serious adverse events or acute GVHD, and had not used corticosteroids. Based on the good safety outcomes reported in this study, assessment of CMVPepVax in a broader and higher risk population of patients undergoing transplantation—eg, those needing high-dose steroids—is planned, to investigate the generality of the current results.

CMVPepVax was designed to stimulate clinically important T-cell subsets, based on the observation of massive expansion of pp65-specific T cells in patients undergoing HCT who have CMV infection.\textsuperscript{19,22} To our knowledge, CMVPepVax is the first CMV vaccine that achieved three major outcomes when used in CMV-seropositive patients who had HCT—ie, a substantial rise in concentrations of pp65 495–503-specific CD8 T cells 100 days after HCT, reduced incidence of CMV reactivation, decreased use of antiviral drugs, and increased relapse-free survival. The outcomes recorded with CMVPepVax suggest that humoral immunity is not required for control of CMV viraemia after HCT.\textsuperscript{24,27} Thus, incorporating humoral targets—eg, CMV glycoprotein B in the ASP0113 vaccine\textsuperscript{13}—is probably unnecessary in the design of a protective CMV vaccine for the HCT setting. The pp65 495–503 epitope contained in CMVPepVax has been identified and characterised by our research group and others, along with a repertoire of HLA MHC class I epitopes within the pp65 protein that can efficiently expand human pp65-specific memory cytotoxic CD8 T cells in vitro.\textsuperscript{15,18,28} Importantly, reconstitution of CMV pp65 495–503-specific cytotoxic CD8 T cells after HCT correlates with protection from CMV and improved outcome of CMV disease.\textsuperscript{1} Moreover, using HLA-restricted CD8 T-cell epitopes to develop a non-infectious subunit CMV vaccine can eliminate the concerns of patients undergoing HCT about the safety of live-attenuated CMV or recombinant live viral vaccines while avoiding the many CMV-encoded proteins entailed in immune evasion.\textsuperscript{3} In CMVPepVax, the HLA-A*0201 pp65 CD8 T-cell epitope is linked covalently to a potent recall antigen activating CD4 T cells—a native tetanus T-helper cell epitope that is recognised by many HLA-DR alleles, with potential to support expansion of CMV pp65-specific CD8 T cells.\textsuperscript{15} Findings of a trial in patients with glioblastoma showed that preconditioning the vaccine site with tetanus toxoid helped to improve survival outcomes of patients receiving a CMV pp65-specific dendritic cell vaccine.\textsuperscript{29} In CMVPepVax, presence of the tetanus toxin T-helper epitope—fused with the CD8 T-cell epitope—could sustain the CMV-specific immune response\textsuperscript{29} while the TLR9 agonist adjuvant has the role to stimulate cellular immunity.\textsuperscript{18,28} Activation of TLR9-expressing cells with PF03512676 induces systemic T-helper 1-like immune effects, which can be considered in two stages: an early innate immune activation and a later enhancement of adaptive immune responses.\textsuperscript{23}
Articles

Study findings showed that CMV-specific T cells generated in vitro from CMV-seronegative donors, who do not have CMV-specific memory T cells, do not recognise the pp65\textsubscript{495-503} epitope, by contrast with CMV-specific T cells from most HLA-A\textsuperscript{*}0201-positive and CMV-seropositive donors.\textsuperscript{7} Yet, data from our trial indicated that significant expansions of pp65\textsubscript{495-503}-specific CD8 T cells were reached in patients who received the CMVPepVax vaccine from both CMV-seronegative and CMV-seropositive donors. In patients assigned the CMVPepVax vaccine in our study, the naive T-cell repertoire from the CMV-seronegative donor could potentially diversify and expand because of both subclinical CMV viraemia and presence of the pp65-based vaccine. Atypical CMV pp65 epitope recognition has been reported\textsuperscript{4} that could also be attributable to the in-vitro methods used to generate CMV pp65-specific T cells, which might not fully reflect the actual kinetics of CMV-specific T-cell expansion in the HCT setting.

In our study, vaccine immune monitoring was done with pentamer technology, which is a useful method for prediction of recurrent or persistent CMV infection in patients undergoing allogeneic HCT.\textsuperscript{21} However, other investigators have assessed intracellular cytokine production after CMV-specific T-cell stimulation and found that the inability to control CMV reactivation might also be related to impaired function of antigen-specific CD4 and CD8 T cells.\textsuperscript{22} Thus, the absence of functional data related to the CMVPepVax response is a limitation of this study.

A limitation of our vaccine is that CMVPepVax can be administered only to patients positive for the HLA-A\textsuperscript{*}0201 allele. This allele is expressed by about 40% of the population; findings suggest that 90% coverage of all major ethnic groups is attainable with 15 uniquely defined HLA-restricted epitopes.\textsuperscript{23} CMV pp65 epitopes restricted to other major HLA types have been well characterised,\textsuperscript{24} and in humanised transgenic mouse models, we have verified the immunogenicity of several promising HLA-restricted pp65 chimeric peptides, including HLA-B7, HLA-A1, and HLA-A11 (unpublished data).\textsuperscript{31} These data could be used to produce a universal multipeptide pp65 vaccine.\textsuperscript{25,26} Patients positive for both HLA-A\textsuperscript{*}0201 and HLA-B\textsuperscript{*}0702 were not specifically excluded in our present study. Previous findings from our group indicate the relative immunodominance of HLA-B\textsuperscript{*}0702 pp65 CD8 T-cell responses in individuals sharing HLA-A\textsuperscript{*}0201 and HLA-B\textsuperscript{*}0702 alleles.\textsuperscript{27} In these patients, concentrations of CD8 T cells specific for the HLA-B\textsuperscript{*}0702 pp65\textsubscript{417-426} epitope were higher than those specific for the HLA-A\textsuperscript{*}0201 pp65\textsubscript{495-503} epitope.\textsuperscript{29} Because of the wide variability of concentrations of CMV-specific CD8 T cells in CMV-seropositive individuals,\textsuperscript{23} we did not exclude patients positive for both HLA-A\textsuperscript{*}0201 and HLA-B\textsuperscript{*}0702 from receiving a vaccine targeting the HLA-A\textsuperscript{*}0201 pp65\textsubscript{495-503} epitope, even if the response to a future vaccine containing the HLA-B\textsuperscript{*}0702 pp65\textsubscript{417-426} epitope could be stronger. None of the four patients positive for HLA-A\textsuperscript{*}0201 and HLA-B\textsuperscript{*}0702 who were assigned observation reactivated CMV. No patients positive for both HLA-A\textsuperscript{*}0201 and HLA-B\textsuperscript{*}0702 were assigned the vaccine.

The favourable clinical outcomes noted in this report were from a randomised—albeit single-institution and non-blinded—trial. Clearly, these results need to be confirmed in a larger, placebo-controlled, multicentre trial. Despite this limitation, the observations we noted were based on analysis of hard data events—ie, reduction in CMV reactivation, decrease in use of antiviral therapy, and relapse-free survival. Although the study population size was small, statistical analysis showed that CMV reactivation and the number of days of antiviral treatment were higher under observation than in patients assigned the vaccine. A possible limitation of our study is the one patient assigned vaccine who reactivated CMV, in which the treating clinician elected to discontinue oral valganciclovir earlier than stated in the institutional (and protocol-specified) standard of care, because of the patient’s renal insufficiency and pancytopenia, although viraemia was resolved despite the short course of treatment. Furthermore, our trial was phase 1b, therefore it was not designed to assess treatment efficacy; but, unexpectedly, we reported significance values not inferior to those of phase 2 oncology trials, which are commonly designed around one-sided type I error rates of 0.10.\textsuperscript{14}

The assay detection limit for CMV viraemia at our institution was 500 gc/mL. Subclinical undetected reactivation could have happened and boosted pre-existing immunity in patients undergoing HCT. However, our study was randomised; thus, there was an equal chance of subclinical reactivation occurring in both study groups. Early CMV reactivation, CMV viral load, and subsequent marrow suppression, lymphopenia, and CMV-specific T-cell immunodeficiency induced by antivirals are predictors of late CMV disease and death after HCT.\textsuperscript{15} Study findings showed CMV reactivation was detrimental to the integrity and heterogeneity of the reconstituting T-cell repertoire in patients receiving a transplant from a matched-unrelated donor.\textsuperscript{30} Moreover, prevention of CMV reactivation could profoundly improve immune reconstitution after HCT.\textsuperscript{21}

The prolonged haematological relapse-free status seen in patients assigned the vaccine is noteworthy, but its interpretation requires caution and can be best validated by expanding accrual to a larger population of patients undergoing HCT, in a placebo-controlled phase 2 trial. Although many factors affect the outcome of allogeneic HCT, disease type and disease status at the time of transplantation are the strongest determinants of survival after the procedure. The Disease Risk Index was developed and has been applied successfully to stratify disease risk across various histologies and allogeneic
HCT regimens. Importantly, in our population, the index was balanced between study groups, even if patients were heterogeneous in terms of underlying disease. Although a complete longitudinal analysis after HCT was not done (which is a limitation of our study), duration of immunosuppressive treatment did not differ between study groups during the period after HCT. Thus, it seems unlikely that differences in clinical course or duration of immunosuppressive treatment among patients have affected the clinical outcomes, although we cannot completely exclude this possibility.

Stimulation of TLR9-expressing cells through the PF03512676 adjuvant could increase amounts of natural killer (NK) cells, the first lymphocytes to reconstitute after HCT, which can limit herpes virus infection, increase the grafted-versus-leukaemia effect, and diminish GVHD. A population of highly cytotoxic NK2G2+ NK cells has been linked to CMV reactivation and might have a role in protecting against relapse after HCT. CMV PepVax could sustain an immunological milieu similar to that cultivated by CMV reactivation, enhancing relapse-protective innate immunity, but without CMV-associated morbidity. NK and NK2G2+ cells were not assessed in this study, but this analysis will be part of a phase 2, multicentre, placebo-controlled trial of CMV PepVax with follow-up to 365 days after HCT, which is currently accruing patients (ClinicalTrials.gov, NCT02396134). If beneficial clinical outcomes are confirmed, CMV PepVax might provide a safe and effective immune-stimulating treatment for CMV-seropositive recipients in the early stages after HCT, when patients are at enhanced risk for CMV reactivation.

References


