Cytomegalovirus chimeric epitope vaccine supplemented with PF03512676 (CMVPepVax) in allogeneic hematopoietic stem cell transplantation: viremia, immunogenicity and survival outcomes in a randomised phase 1b trial

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Summary

Background—Cytomegalovirus (CMV) seropositive recipients of allogeneic hematopoietic cell transplantation (HCT) are at risk for CMV reactivation. Stimulating viral immunity by vaccination may achieve CMV viremia control, without the need for antivirals. The aim of the trial is to assess safety, immunogenicity, and possible clinical benefit of CMVPepVax vaccine in HCT recipients.

Methods—In this randomised, open-label phase 1b trial, HCT recipients were enrolled at a single USA transplant center. Eligible patients were CMV seropositive, HLA A*0201-positive,

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18–75 years, receiving HCT from matched related or unrelated donors. Patients were reassessed on day 28 post-HCT for eligibility, and 36 patients were randomised either to the vaccine (VA) or observation arm (OA), in blocks stratified by CMV donor serostatus. CMVPepVax was administered subcutaneously on days 28 and 56. 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 (Pfizer Inc) a Toll-like receptor 9 agonist, which augments cellular immunity. The primary outcome was safety; secondary outcomes included immunogenicity, prevention of CMV reactivation, and clinical outcomes. Statistical analyses included all 36 randomized patients and were performed as per protocol. This study is registered as NCT01588015@www.clinicaltrials.gov. This trial is closed to accrual and a final analysis is presented in this report.

Findings—Between October 31, 2012, and November 5, 2014, 36 HCT recipients were randomised into the study. CMVPepVax was administered to 18 patients, with no adverse effect on HCT or rate of acute GVHD, and no unexpected adverse events. One serious adverse event (grade 1 fever) was attributed to CMVPepVax vaccination and resolved within 48 hours. Higher relapse free survival (1 versus 7 events, logrank p=0·015), a 2 fold increase in CMV-pp65 CD8 T cells during the first 100 days post-HCT (p=0·025), less CMV reactivation (1 versus 6 events, logrank p=0·039) and usage of antivirals (15 versus 263 days, p=0·03) were found in VA compared to OA recipients.

Interpretation—The results demonstrate safety and immunogenicity of CMVPepVax, and the prospect of significant clinical benefits that warrant testing in a phase 2 trial.

Introduction

Allogeneic hematopoietic stem cell transplantation (HCT) has curative properties for many hematologic disorders. Early post-HCT, both innate and adaptive immunity are impaired, due to immunosuppression associated with the procedure. As a result, HCT recipients are highly susceptible to opportunistic infections. Despite preemptive antiviral therapy, cytomegalovirus (CMV) remains the leading infectious complication in HCT recipients. CMV reactivation primarily occurs within the first 100 days post-HCT, in more than one third of CMV-seropositive patients, the group at highest risk for CMV reactivation. Due to early CMV reactivation post-HCT and enhanced risk of severe end-organ disease, CMV positive serology, either of the donor or the recipient remains associated with higher non-relapse mortality and poorer overall survival. Current antiviral therapy effectively limits viremia, however its use is associated with systemic and organ toxicity, which besides adding to the cost of HCT, creates delays in immune reconstitution, increases fungal/bacterial infections, breakthrough gastrointestinal CMV disease, and risk of late-onset CMV disease.

Immunotherapy based on infusion of limited numbers of CMV specific T cells was found to promote restoration of durable, functional antiviral immunity, which effectively bridges the early post-HCT period of high susceptibility to uncontrolled CMV viremia. In particular, adoptive therapy of HLA restricted CMV pp65 T cells resulted in successful treatment of CMV infection not responding to antivirals. The pp65 tegument protein is among the most frequently recognized CMV antigen in CMV seropositive healthy adults. A recent

investigation has also shown the feasibility of generating pp65-specific T cells from CMV naïve donors for effective immunoprophylaxis. Despite the success of pp65 cell infusion approaches, there are hurdles for employing adoptive T-cell therapy for general use. Exploiting natural immune response mechanisms by therapeutic vaccination during the periods of greatest risk post-HCT is a feasible approach to control CMV infection. Vaccine driven responses may be challenging to elicit early post-HCT, since the recipient's immune system remains impaired for the first months post-HCT. Thus, vaccination for preventing infectious diseases in HCT recipients are generally recommended to begin no earlier than 6 months post-procedure, well after the period of highest risk for CMV infection. Nonetheless, recent studies have indicated that recovery of pp65 CD8 T cells during the first 65 days post-HCT is associated with protection from CMV related complications. A recent clinical trial in HCT recipients has shown that TransVax vaccine (renamed ASP0113; Astellas Pharma Inc, Tokyo, Japan) was safely administered early post-HCT. ASP0113, a CMV DNA vaccine containing plasmids encoding for pp65 and the surface glycoprotein B (gB) failed its primary endpoint of reduction in CMV viremia requiring antiviral therapy, although time-to-first episode of viremia was longer, and rates of CMV viremia were lower in the ASP0113 vaccinated recipients.

The continuing unmet need for a CMV vaccine in the HCT setting, prompted the 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 (TLR) 9 agonist, PF03512676, as an adjuvant just prior to patient administration. An acceptable safety profile and vaccine-driven expansion of pp65 T cells in healthy adults when used with PF03512676 supported its further evaluation in HCT recipients. The objective for the CMVPepVax vaccine is to stimulate a CD8 T-cell response directed towards $pp65_{495-503}$, a dominant T-cell epitope of the pp65 tegument protein involved in CMV viremia protection early post-HCT. The objective of this study was to assess safety, immunogenicity and clinical benefit of CMVPepVax, in a CMV seropositive cohort of patients at high risk for CMV reactivation and end-organ disease.

Methods

Study design

This single site, randomised, open label, parallel arm, phase 1b trial was conducted at City of Comprehensive Cancer Center (COH), Duarte, CA, USA. The study was approved by the local institutional review board (IRB 12022), and the Food and Drug Administration (FDA, investigational new drug BB-13124, held by COH) before initiation of enrollment.

Patients

We recruited patients from among those scheduled to undergo allogeneic HCT for hematologic malignancies at COH. According to COH standard of care (SOC), HCT is permitted in patients with 60% Karnofsky performance status, and >3 months estimated survival. All participants signed an IRB approved, written informed consent, and were eligible if they were CMV seropositive, HLA A*0201, 18–75 years old, willing to be

monitored at least for 6 months, and having either a related or unrelated donor with 8/8 or 7/8 (A,B,C,DRB1) high resolution HLA donor allele matching. During the study period of 10/2012 to 11/2014, a little over 2/3rd of HCT patients were CMV seropositive. At our institution 34% of these were HLA A*0201 positive, and candidates for the current CMVPepVax. We identified 130 patients from this target population (555 HCT patients $\times$ 0.69 CMV seropositive $\times$ 0.34 HLA A*0201= 130 patients) for further screening (Figure 1). Further eligibility restrictions were applied for the sake of safety and to facilitate follow-up in this first trial of CMVPepVax in HCT recipients. Of 130 patients, 68 were deemed ineligible due to the following: age (n=2), diagnosis (aplastic anemia, myeloma (n=4), current/recent CMV infection (n=2), cord blood (n=1), history of autoimmune disease (n=5), hepatitis B, C, or HIV infection (n=2), HLA mismatch (prior to the protocol amendment to allow 7/8 match after 7 months of accrual; n=8), not in complete remission (CR)/beyond second CR (n=26), medical/psychosocial comorbidities that the treating physician did not agree with the trial (n=18). Sixteen patients were considered eligible but self-declined or the study was not offered to them due to staff limitations, which prevented the single research nurse responsible for all HCT protocols from approaching all potential patients. Use of myeloablative, reduced intensity, and non-myeloablative conditioning regimens were permitted pre-transplant. Conditioning regimen selection followed COH standard treatment/ practice guidelines. Fully ablative regimens included fractionated total body irradiation (TBI: 1200cGy) plus etoposide (60 mg/kg) or cyclophosphamide (100 mg/kg). Reduced intensity regimens were nearly ablative as they included melphalan (100–140 mg/m^2) in combination with fludarabine (125 mg/m^2) or clorafabine (30–40 mg/m^2), resulting in >90% donor T cell engraftment by day 30 in most of our patients. Rituximab was allowed in cases of B cell lymphoma. GVHD prophylaxis consisted of tacrolimus and sirolimus.

The duration of immunosuppressive therapy (IST) was according to the institutional SOC and tapering of IST typically started on day 100 post-HCT, in the absence of GVHD. Thus, patients in both arms were under full IST coverage during the first 100 days post-HCT. There was no difference in the incidence of acute or chronic GVHD between arms, furthermore IST was also similar between them. In detail, median prednisone daily dose over the first 56 days (data collected as specified per protocol) was 382·5 mg (interquantile range [IQR]=0–776·2) for the OA, and 535 mg for the VA (IQR=192·5–820); median tacrolimus daily dose was 62·5 mg (IQR=57·25–76·5) for the OA, and 93·5 mg for the VA (IQR=64–115·2); median sirolimus daily dose was 95 mg (IQR=60–148·2) for the OA, and 164 mg for the VA (IQR=116–228·8). Additionally, at day 100 and 180 median prednisone daily dose was 0 (IQR=0–10) and 3·75 mg (IQR=0–10), respectively for the OA; and 2·5 (IQR=0–10) and 0 mg (IQR=0–8·75), respectively for the VA. A short course of methotrexate (5 mg/m^2 on days 1, 3, and 6 post-HCT, or 15 mg on day 1 post-HCT, and 10 mg/m^2 on days 3, 6, and 11 post-HCT) was given to patients receiving HCT from a 7/8 matched donor. No patients received T-cell depleting agents (e.g. antithymocyte globulin) or an ex-vivo T-cell-depleted graft. The donor T cell median around day 30 post-HCT was 97·9% (range 86·4–100%) in the OA group, and 97·8% (range 73·8–100%) in the VA group. Thus impact of residual host T cells was negligible. Supportive care, including prophylactic antibiotics, antifungal therapy, total parenteral nutrition, hematopoietic growth factors, immune globulin replacement, and treatment of mucositis and neutropenic fever was

administered in accordance with institutional standard practice guidelines. All patients received herpes simplex virus/varicella zoster virus prophylaxis using standard-dose acyclovir. Preemptive management of CMV infection was allowed, but any planned use of prophylactic anti-CMV antivirals or CMV immunoglobulin was prohibited. Additional exclusion criteria included receiving T-cell depleted HCT, aplastic anemia, acute leukemia not in remission, receipt of a live-attenuated vaccine within 30 days post-HCT, previous therapy for CMV viremia, congenital or acquired immune deficiencies, autoimmune disease, HIV, hepatitis C and active hepatitis B positivity (surface antigen negative). On day 28 post-HCT, each enrolled patient was reassessed for eligibility and excluded 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 500/mm^3), experienced CMV reactivation [500 genomic viral copies (gc)/mL], had grade III–IV (according to the Keystone Consensus grading system) acute (a) GVHD, received steroids >1 mg/kg/day within 7 days of immunization, or had any ongoing non-hematologic toxicity grade 3 (graded according to the Common Terminology Criteria for Adverse Events, CTCAE v3·0).

Randomisation, and procedures

On day 28 post-HCT, a computer-generated randomisation, in blocks of 4, stratified by CMV donor serostatus, with a ratio of 1:1 assigned each eligible recipient either to the vaccine arm (VA; n=18) or to the non interventional, observation arm (OA; n=18). The registrar concealed assignments until consent and eligibility at day 28 were established. In the VA, eligible recipients were injected twice (on days 28 and 56 post-HCT) subcutaneously (s.c.) with CMVPepVax. The National Cancer Institute sponsored Rapid Access to Interventional Development program contracted with Bachem (Torrance, CA, USA) for current good manufacturing practices (cGMP) grade production of the peptide portion of the CMVPepVax vaccine. The cGMP-grade peptide vaccine (NSC-721434) consists of the HLA A*0201 specific pp65$_{495-503}$ (NLVPMVATV) CD8 T-cell epitope fused with the P2 epitope of tetanus toxin (tt$_{830-843}$: QYIKANSKFIGITE).· The adjuvant, PF03512676 is a synthetic single-stranded phosphorothioate DNA-containing CpG motifs (24 nucleotides in length), that was supplied by Pfizer Inc (New York, NY, USA), and is classified as an investigational agent. CMVPepVax vaccine formulation is comprised of 2·5 mg of peptide vaccine solution and 1·08 mg PF03512676, in a final 1 ml injection volume (Figure 1, web extra material). In the CMVPepVax healthy volunteer trial four doses of the vaccine were administered 3 weeks apart to assess safety of multiple injections. Since it was unknown whether the use of an agent augmenting cellular immunity, such as PF03512676 could have impacted the development or severity of aGVHD, HCT patients received only 2 doses of CMVPepVax, Additionally a four week interval was chosen to fully assess any toxicities attributable to the first vaccine dose, including development of aGVHD. The vaccine was delivered s.c. in the upper arm. For patients with incomplete hematopoietic reconstitution, who were at risk for deep vein thrombosis, s.c. is considered the safest route. Additionally, it is the optimal route for PF03512676 activation of innate immunity.·

GVHD and adverse events (AEs) were monitored for both VA and OA participants as necessary and not less than bi-weekly from day 28 until day 100 post-HCT. Afterwards,

GVHD was monitored as necessary or monthly until 6 months, and subsequently as per SOC.

CMV viremia was assessed by polymerase chain reaction (PCR) methods using quantitative (q) PCR, with probes designed to detect the UL83 and UL55 genes of the CMV genome. Further details are proprietary to COH and/or Focus Diagnostics (Cypress, CA, USA), but meet USA CLIA requirements for clinical diagnostic laboratories. The current assay from Focus Diagnostics is FDA approved (Simplexa™ CMV Kit). Quantitative results were reported between 500 to 500000gc/mL (1gc/mL≈2·5IU/mL). This test was developed and its performance characteristics determined by the COH Clinical Molecular Diagnostic Laboratory, which is certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988. Monitoring for CMV viremia by qPCR was performed twice a week from day 21 to 100 post-HCT, and thereafter a rigorous patient-based and risk-adapted monitoring was implemented, per institutional SOC. Approximately 40% (14/36) of the total study population was tested 10 times or more between day 100 and day 180, with some patients still monitored bi-weekly through day 180. The median number of PCR assays past day 100 was 7 on the VA (IQR=3–10) and 7.5 on the OA (IQR=4·5–11·5).

According to COH SOC, matched related or unrelated HCT patients received pre-emptive anti-CMV therapy (ganciclovir, valganciclovir or foscarnet) when a CMV PCR value became 1500gc/ml. Preemptive therapy was recommended for CMV PCR <1500gc/ml only when patients were considered at high risk for CMV disease, such as when receiving high-dose steroids (see "Preemptive therapy" in web extra material).

Occurrence of CMV disease, and number of CMV specific antiviral treatment days were recorded up to 6 months. Hematologic relapse and death were monitored until May 31, 2015. As required by the FDA, since the CpG-DNA portion of the CMVPepVax vaccine has been associated with autoimmunity, double strand (ds) DNA IgG autoantibody were measured using Wampole® DS DNA ELISA II (Alere, Orlando, Florida, USA), in the serum of vaccinated recipients at days 28, 56, 100, 180 post-HCT.

CMV-specific immunogenicity was monitored in peripheral blood mononuclear cells (PBMC) of all enrolled recipients (n=36) every 2 weeks from day 28 until day 100 post-HCT, then at days 130, 160 and 180, by measuring levels of CD8 T cells binding to MHC class I $pp65_{495-503}$ and $HIVgag_{77-85}$ pentamers (ProImmune, Oxford, UK). Percentages of binding to $HIVgag_{77-85}$ pentamers, used as a background control were subtracted. The assay lower limit of detection was 0.02% of CD8 T cells (frequency) or 0.1cell/µl (absolute cell count). PBMC for each time point were labeled and analysed by fluorescence-activated cell sorting (FACS; Gallios™, Beckman Coulter with Kaluza analysis software, Brea, CA), as previously detailed.

Outcomes

The primary outcome of the trial was to evaluate the safety of CMVPepVax in HCT recipients. The key safety endpoints were: secondary graft failure, grade III–IV aGVHD, day 100 non relapse mortality (NRM), serious adverse events (SAEs) related to the vaccine, grade 3–4 related AEs (CTCAE) within 2 weeks of vaccination, and development of anti-

dsDNA auto-antibodies. Safety of all patients was monitored on an ongoing basis up to day 180 post-HCT.

The secondary objectives were to assess CD8 T-cell response to pp65$_{495-503}$ in VA versus OA patients. Additional endpoints included clinical/laboratory evaluation of CMV reactivation and disease, aGVHD, chronic GVHD, relapse-free survival (RFS), non-relapse mortality (NRM), relapse rate in VA compared to OA HCT recipients.

Statistical analysis

All 36 randomised patients were included in the analysis of clinical outcomes. Clinical data were analyzed "as randomized," while the analysis of T-cell response focused on patients who did not reactivate CMV. Of the VA patients (n=18), four did not qualify to receive the 2nd CMVPepVax injection, and of the OA patients (n=18) four reactivated before day 56, and would have been ineligible for vaccine injection. Also, four OA patients were followed for less than 180 days post-HCT: three due to relapse, and one who relocated at considerable distance for COH, and withdrew consent for further blood draws at day 130 post-HCT (Figure 1).

Safety evaluation was based on the protocol-specified monitoring rules, and the summary of AEs. Differences between arms for SAE and AE were compared by two-sided rank-sum test, and Fisher's exact test. The cumulative incidence of CMV viremia was estimated by the product-limit method. Proportional hazard (Cox) models were fit to estimate hazard ratios, with significance levels based on log-rank tests, and checked for qualitative agreement with likelihood ratio tests. Similar methods were used to compare relative hazard of other events across arms. The main evaluation of immunogenicity omitted patients with CMV reactivation (6 on OA, 1 on VA), in an attempt to distinguish the effect of vaccination from that of exposure to replicating CMV. A protocol specified analysis compared levels of CD8 T cells specific for CMV pp65$_{495-503}$ in VA and OA recipients by Wilcoxon rank-sum test, using integrated post-vaccination CMV pp65$_{495-503}$-specific CD8 T-cell levels through day 100 post-HCT, as a numerical outcome. This was done on a logarithmic scale, to represent individual responses relative to baseline levels (day 28 post-HCT), which had highly variable and skewed distributions, characteristic of the wide variability of post-HCT immune reconstitution timing. The trial had a planned 90% power for the rank-sum test to detect a vaccine effect that would place about 90% of VA recipients at CMV pp65$_{495-503}$-specific CD8 T-cell levels characteristic of the upper 50% of OA recipients, a shift such that 4 equally likely intervals on the OA would be occupied with probabilities of 4, 8, 17, and 71 percent, respectively (proportional odds model). As a more probative analysis, generalized estimating equation (GEE) methods were used to model the marginal means, on a log scale, as functions of vaccination, baseline CMV pp65$_{495-503}$-specific CD8 T-cell levels, time post-HCT, and donor CMV serostatus. Computing was done using R version 3·1·2 with the survival (T.M. Therneau, version 2·37–7) and gee (V.J. Carey, version 4·13–18) packages. Patients were censored at relapse with respect to assessment of CMV reactivation and non-relapse mortality.

COH Data and Safety Monitoring Committee (DSMC) reviewed and monitored toxicity and accrual data from this trial. The Committee was composed of COH clinical specialists with

experience in oncology, and who had no direct relationship with the study. The DSMC reviewed up-to-date participant accrual; summary of all AEs 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 COH Office of Clinical Trials Auditing and Monitoring.

This trial has reached time of patient follow up, and was registered as NCT01588015@www.clinicaltrials.gov.

Role of the funding source

The National Cancer Institute and Pfizer Inc (the provider of PF03512676 adjuvant) had no role in study design, data collection, analysis, and interpretation, and the final decision for content rests with the authors. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between September 19, 2012, and September 29, 2014, a total of 46 eligible pre-HCT patients were enrolled into the trial. None reactivated CMV by day 28 post-HCT. On day 28 post-HCT, patients were reassessed for eligibility and between October 31, 2012, and November 5, 2014, 36 eligible patients were randomised to VA (n=18) and OA (n=18). Six enrolled patients (6/18; 13%) did not meet eligibility criteria for randomisation into the study at day 28; one patient received steroids >1 mg/kg/day; two patients had grade III aGVHD; three patients had ongoing grade 3 AE. Additionally, three patients withdrew, and one patient died early post-HCT before day 28 randomisation (Figure 1). The eligible 36 randomised patients were included in this report. Randomisation was stratified by donor serostatus (recipients were all CMV seropositive), which led to a close balance between the two study arms (Table 1). All patients received peripheral blood stem cell grafts. Twenty-three recipients (64%; 23/36) had reduced-intensity conditioning, which was comparably distributed between OA and VA, and the remainder had a fully ablative regimen. Patient demographic, clinical characteristics and primary hematologic diagnosis were similarly distributed across arms. As for type of transplant, VA had more matched unrelated (MUD) HCT recipients (11 of 18) than did OA (6 of 18). Despite this difference, Karnofsky score at randomisation and the overall Disease Risk Index (DRI), the strongest determinant of post-HCT survival, was balanced between arms (Table 1).

None of the 18 patients in the VA met the pre-defined stopping-rules after the 1st or 2nd vaccination (secondary graft failure, grade III–IV aGVHD, day 100 NRM, grade 3–4 related AEs within 2 weeks). None of the VA patients required dose reduction; none discontinued the vaccination regimen for drug related toxicity. No vaccine treatment-related deaths were recorded. Follow-up for AEs was targeted for the period from first vaccination (day 28) to day 100 post-HCT, with onset of the recorded AEs ranging from day 23 to 103. For three OA patients, follow-up was curtailed as per protocol by relapse at days 65, 77, and 97 respectively (Figure 1). One OA patient relocated and withdrew consent at day 130 for further blood draws, and follow-up for RFS continued (Figure 1). For one VA patient, a single SAE attributed to CMVPepVax vaccination consisted of a grade 1 fever on the day of

vaccination, requiring hospital admission, and resolution of fever occurred within 48 hours. We observed unrelated SAEs in 4 VA patients (maximum grades 3, 3, 2, and 1 respectively), whereas SAEs were reported in 9 OA patients (1 with grade 4, and 4 with maximum grade of 3). SAEs and AEs grade 3–4 did not differ between arms (Table 2). In contrast to the healthy volunteer trial in which injection site reactions were commonly reported with CMVPepVax, local and systemic skin reactions were negligible in the HCT VA cohort. Among the four patients who did not receive the 2nd vaccine injection, two patients became ineligible per protocol, since CMV antiviral therapy was required; one patient self-declined the 2nd vaccine injection; one patient had ongoing grade 3 toxicities related solely to the HCT procedure, and the treating physician decided to withhold the 2nd vaccine injection. No VA patients developed anti-dsDNA auto-antibodies after CMVPepVax injections, up to 6 months post-HCT (Figure 1, web extra material).

The incidence of aGVHD between the two arms was similar: aGVHD occurred post-randomisation in 6/18 subjects (grade I: 1 patient, II: 5 patients) for the OA, and in 7/18 subjects (grade I: 1 patient, II: 6 patients) in the VA. There was no grade III-IV aGVHD after CMVPepVax immunizations (Table 2). Chronic GVHD had similar incidence in both arms. CMVPepVax did not adversely affect survival. All 18 patients in the VA were alive as of May 31, 2015, including one who developed relapse of their underlying disease ~17 months (531 days) post-HCT. In contrast, 5 hematologic malignancy relapses and 7 deaths (5 due to relapse) were observed in the OA (Table 2) compared to the VA (Figure 2; relative hazard=0·12, p=0·015, two-sided likelihood ratio test; 95% confidence interval [CI]=0·01–0·94)).

Figure 3 summarizes individual time courses of $pp65_{495-503}$-specific CD8 T-cell levels after randomisation (day 28 post-HCT), in VA and OA recipients who did not subsequently have CMV reactivation. As expected, there was wide variability in T-cell levels in both trial arms (Figure 3 web extra material). Nonetheless, the VA exhibited a 3.5-fold increase (95% CI=1·6–7·9; p=0·0018) in geometric mean from vaccination (day 28) to day 100 post-HCT, compared to 1.4-fold over baseline (95% CI=0·6–3·3; p=0·27) for the OA in the same period (by piecewise-linear GEE models on a log-scale). The protocol-specified rank-sum test (see Methods) indicated a statistically significant vaccine effect (2 fold greater rise from baseline in VA relative to OA, averaged to day 100; p=0·025; 95% CI=1·04–3·51), as did direct comparison of arms in GEE models (2·5 fold greater increase from baseline to plateau beyond day 100, p=0·046). Donor CMV serostatus did not significantly impact $pp65_{495-503}$-specific CD8 T-cell levels. In CMV seropositive recipients, $pp65_{495-503}$-specific CD8 T-cell levels were on average 23% higher (SE +/− 42%) than in CMV seronegatives (p=0·57). Highly variable pp65-specific T-cell profiles were found among viremic patients, possibly due to the different post-HCT times/levels of CMV reactivation and length/type of antivirals administered. After viremia resolution, pp65-specific T-cell levels generally increased in the viremic patients, but did not exceed levels measured in the VA patients, who did not reactivate.

In UPN 7, the only VA patient in whom CMV reactivated, a modest increase in $pp65_{495-503}$-specific CD8 T-cells (0.43 cells/μl) on day 42, 15 days after the first vaccination was detected. Treatment with antivirals started on day 47, leukopenia (WBC 1.9 k/μl) and

concomitant drop of pp65$_{495-503}$-specific CD8 T-cells to detection limits were observed on day 56. In contrast, a substantial increase in pp65$_{495-503}$-specific CD8 T-cells was observed after day 100 (>2 cells/μl).

CMV reactivated in seven enrolled patients, six reactivated before day 100, and one experienced a late CMV reactivation (day 150). Rates of CMV reactivation in the VA were significantly lower compared to the OA. We observed only 1 VA patient (D$^-$/R$^+$) who had detectable viremia levels (500 gc/mL, starting at day 47 post-HCT), compared to 6 CMV viremic patients in the OA (Table 2, Figure 4, p=0·039, two-sided log-rank; 95% CI=0·02–1·1). Reactivation times for the OA patients were as follows, UPN 6: day 35; UPN 12: day 38; UPN 15: day 95; UPN 19: day 146; UPN 27: day 37. In one OA patient, after a preceding CMV viremia, gastrointestinal (GI) CMV disease developed, with both positive CMV histopathology and tissue culture evidence of virus at day 130 post-HCT. In one VA patient, (D$^+$/R$^+$) GI endoscopy was performed due to GI aGVHD: *Clostridium difficile* was isolated, and CMV histopathology was found to be positive on day 40 post-HCT. This patient did not develop CMV viremia (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 viremia observed in this patient was an effect of the 1st CMVPepVax injection. The treating physicians decided to provide antiviral treatment for 60 days for a presumptive diagnosis of CMV gastroenteritis;· consequently the 2nd vaccine injection was not administered as per protocol.

Duration of pre-emptive antiviral administration significantly differed between arms. The 6 OA patients in whom CMV reactivated spent a total of 263 days on antivirals (138 days of induction doses, and 125 days of maintenance) compared to 15 days (7 induction, 8 maintenance) for the single VA patient who became CMV viremic (p=0·039, two-sided rank-sum test; Table 2). As for the only VA patient who developed CMV viremia, CMV PCR levels became undetectable 4 days after treatment began, and since the patient developed renal insufficiency and pancytopenia, oral valganciglovir was discontinued earlier than signified in our institutional SOC (2 weeks of induction and 2 weeks of maintenance). All other patients followed antiviral treatment regimens as per SOC.

Discussion

CMVPepVax, a novel CMV peptide vaccine formulated with PF03512676 adjuvant, a TLR9 agonist was found to be safe and well tolerated in the HLA A*0201 CMV seropositve HCT recipient population studied in this trial. Compared to the OA, patients immunized with CMVPepVax reconstituted significantly higher levels of CMV-pp65 specific CD8 T cells during the first 100 days post-HCT, had reduced CMV reactivation, usage of antivirals and increased RFS (Table 2, Figure 2, 3 and 4).

CMV reactivation remains the cause of major health complications, profound defects in immune reconstitution, and significant morbidity in the recovery of immune-compromised HCT recipients, diminishing the full curative potential of this successful cancer therapy.· This trial was designed to assess safety and explore the clinical outcomes of CMV seropositve HCT recipients receiving the CMVPepVax vaccine, who are at enhanced risk for

CMV reactivation, and most likely to be administered antivirals. Since CMV reactivation is mainly controlled by CMV-specific T cells, vaccine injections were administered with the intent of eliciting a protective immune response preceding CMV reactivation in the CMVPepVax immunized recipients. Data from our group and a recent analysis from the Center for International Blood and Marrow Transplant Research (CIBMTR) indicated that the median time to CMV reactivation for HCT is ~40 days (1–362 days), with 98% of reactivations occurring before day 100, thus our vaccine dosing schedule (days 28 and 56 post-HCT) directly targets the period of greatest CMV reactivation risk post-HCT. In the current study, none of the 36 patients enrolled into the trial reactivated before day 28 post-HCT. The timing chosen for the first immunization seems adequate based on both safety and favorable clinical outcomes. It allows patients to have sufficient time to engraft and recover from acute toxicities from HCT. Additionally, in a prospective study from our group timing of the initiation of the measurable CMV-specific adaptive immune response in allogeneic HCT recipients was strengthened on day 28 post-HCT. Consequently, vaccination at earlier times post-HCT might result in lack of efficacy because of limited hematopoietic reconstitution. Based on the CIBMTR data, follow up to day 180 is consistent for a phase 1b trial, but we recognize the possible limitation.

This was the first use of CMVPepVax in HCT recipients, thus strict eligibility rules were applied (see Methods), consequently only 1/3 of the target population of CMV seropositive and HLA A*0201 type recipients was enrolled, which is a limitation of this study (Figure 1). Additionally, re-assessment at day 28 post-HCT ensured randomisation into the trial of a patient population who had engrafted, did not reactivate CMV, and had not experienced HCT procedure-related SAEs, aGVHD, and usage of corticosteroids (Figure 1). Based on the excellent safety outcomes reported in this study, evaluation of CMVPepVax in broader and higher risk transplant populations, such as patients requiring high-dose steroids is planned to assess 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 HCT patients undergoing CMV infection. To our knowledge, CMVPepVax is the first CMV vaccine that achieved three major outcomes when used in CMV seropositive HCT patients: a significant rise in pp65-specific CD8 T-cell levels during the critical 100 days post-HCT, reduced incidence of CMV reactivation and usage of antivirals, and increased RFS (Figure 2, 3, 4). CMVPepVax outcomes suggest that humoral immunity is not required for CMV viremia control post-HCT. Thus, incorporating humoral targets, such as CMV gB in the ASP0113 vaccine, is likely 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 characterized by our 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*. Importantly, reconstitution of CMV pp65-specific cytotoxic CD8 T cells post-HCT correlates with protection from CMV and improved outcome of CMV disease. Additionally, using HLA-restricted CD8 T-cell epitopes to develop a non-infectious subunit CMV vaccine can eliminate the safety concerns for HCT recipients of live-attenuated CMV or recombinant live viral vaccines, while avoiding the many CMV-encoded proteins involved in immune-evasion. In CMVPepVax, the HLA A*0201 pp65 CD8 T-cell epitope is covalently linked to

a potent recall antigen activating CD4 T cells: a native tetanus T-helper cell epitope that is recognized by many HLA DR alleles, with potential to support the expansion of the CMV-pp65 specific CD8 T cells. A recent trial in glioblastoma patients showed that pre-conditioning the vaccine site with tetanus toxoid, helped to improve survival outcomes of patients receiving a CMV-pp65-specific dendritic cell vaccine. In CMVPepVax, the presence of the tetanus toxin T-helper epitope, fused with the CD8 T-cell epitope could sustain the CMV specific immune response; while the TLR-9 agonist adjuvant has the role to stimulate cellular immunity. Activation of TLR9-expressing cells with PF03512676 induces systemic T_H1-like immune effects, which may be considered in two stages: an early innate immune activation and a later enhancement of adaptive immune responses.

A recent study showed that CMV-specific T cells generated in vitro from CMV seronegative donors, who lack CMV-specific memory T cells do not recognize the $pp65_{495-503}$ epitope, in contrast to most HLA A*0201 CMV seropositive donors. Yet, data from our trial indicated that significant expansions of $pp65_{495-503}$–specific CD8 T cells were reached in CMVPepVax vaccinated HCT recipients from both CMV seronegative and seropositive donors. In CMVPepVax vaccinated CMV seropositive HCT recipients, the naïve T-cell repertoire from the CMV seronegative donor could potentially diversify and expand due to both subclinical CMV viremia and the presence of the pp65-based vaccine. The atypical CMV-pp65 epitope recognition reported in the recent study could be also due to the in vitro methods used to generate CMV-pp65 specific T cells, which may not fully reflect the actual kinetics of CMV-specific T-cell expansion in the HCT setting.

In this study, vaccine immune monitoring was performed using pentamer technology, which has been shown to be a useful tool for prediction of recurrent or persistent CMV infection in allogeneic HCT recipients. However, other investigators have assessed intracellular cytokine production after CMV-specific T-cell stimulation, and found that the inability to control CMV reactivation may also be related to impaired function of antigen-specific CD4 and CD8 T cells. Thus, the lack 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 HLA A*0201 recipients. The HLA A*0201 type is expressed by ~40% of the population; studies have suggested that 90% coverage of all major ethnic groups is attainable with 15 uniquely defined HLA-restricted epitopes. CMV-pp65 epitopes restricted to other major HLA types have been well characterized, and in humanized transgenic mouse models our group has already verified the immunogenicity of a number of promising HLA restricted pp65 chimeric peptides, including HLA B7, A1 and A11. (La Rosa et al., unpublished data). Thus, these data could be used for the production of a universal multi-epitope pp65 vaccine. HLA A*0201- and B*0702-positive patients were not specifically excluded in this study. Earlier findings from our group indicated the relative immunodominance of HLA B*0702 pp65 CD8 T-cell responses in persons sharing HLA A*0201 and HLA B*0702 alleles. In these subjects, CD8 T-cell levels specific for the HLA B*0702 $pp65_{417-426}$ epitope were found to be higher than those to the HLA A*0201 $pp65_{495-503}$ epitope. Due to the wide variability of the CMV-specific CD8 T-cell levels in the CMV-seropositive human population, we did not exclude HLA A*0201- and B*0702-positive patients to receive a vaccine targeting the HLA

A*0201 pp65$_{495-503}$ epitope, even if a response to a future vaccine containing the HLA B*0702 pp65$_{417-426}$ epitope could be stronger. None of the four HLA A*0201- and B*0702-positive patients reactivated CMV in the OA. They were no HLA A*0201- and B*0702-positive patients in the VA.

The favorable clinical outcomes in this trial were observed in a randomised, but single-institution and non-blinded trial. Clearly, these results need to be confirmed in a larger placebo-controlled, multi-center trial. Despite this, the observations noted were based on analysis of hard-data events, such as reduction in CMV reactivation and use of antiviral therapy, and relapse-free survival (Figure 2, 4 and Table 2). Though the study population size of this phase 1b trial was small, statistical analysis revealed that CMV reactivation and number of days of antiviral treatment were higher in the OA, compared to VA trial participants. A possible limitation of this study is the single case of a VA patient who reactivated CMV, in which the treating physician elected to discontinue oral valganciclovir earlier than per our institutional SOC (and protocol specified), due to the patient's renal insufficiency and pancytopenia, although viremia was resolved despite the short course of treatment. This was a phase 1b trial, thus the study was not designed to assess treatment efficacy, but unexpectedly we got 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.

The assay detection limit for CMV viremia at our institution was 500 gc/ml: subclinical undetected reactivation could have happened and boosted preexisting immunity in the HCT recipients. However, the current study was randomized, thus there was an equal chance of subclinical reactivation occurring in both arms. It has been reported that 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 post-HCT. A recent study has described CMV reactivation as detrimental to the integrity and heterogeneity of the reconstituting T-cell repertoire in MUD HCT recipients. Results from that investigation suggested that preventing CMV reactivation can profoundly improve immune reconstitution post-HCT.

The prolonged hematologic relapse free status, observed in the VA (Figure 2) is of interest, but its interpretation requires caution and can be best validated by expanding the accrual to a larger population of HCT recipients, in a placebo controlled phase 2 trial. Although many factors influence the outcome of allogeneic HCT, disease type and disease status at the time of transplantation are the strongest determinants of post-HCT survival. The DRI was developed and has been successfully applied to stratify disease risk across histologies and allogeneic HCT regimens. Importantly, in our HCT population the DRI was balanced between arms, even if the 36 patients were heterogeneous in terms of underlying disease (Table 1). Though a complete post-HCT longitudinal analysis was not performed (which is a limitation of our study), IST duration did not differ between the two arms, during the post-HCT period observed per protocol (see Results). Thus, it seems unlikely that differences in clinical course or IST duration among patients have influenced the clinical outcomes, though the current study cannot completely exclude this possibility.

Stimulation of TLR9-expressing cells through the PF03512676 adjuvant may increase levels of NK cells, the first lymphocytes to reconstitute post-HCT, which can limit herpes virus infection, increase graft versus leukemia effect, and limit GVHD. Recently, a population of highly cytotoxic NKG2C^{+} NK cells has been linked to CMV reactivation and may have a role in protecting against relapse post-HCT. CMVPepVax might sustain an immunologic milieu similar to that cultivated by CMV reactivation, enhancing relapse protective innate immunity, but without CMV-associated morbidity. NK and NKG2C^{+} cells were not evaluated in this study, but will be part of a phase 2 evaluation of CMVPepVax. This phase 2 multicenter, placebo controlled trial (NCT02396134@www.clinicaltrials.gov.), with a follow up to 365 days post-HCT is currently accruing patients. If beneficial clinical outcomes are confirmed, CMVPepVax would provide a safe and effective immune-stimulating therapeutic for the care of CMV seropositive recipients early post-HCT, when they are at enhanced risk for CMV reactivation, and subsequent antiviral therapies associated with significant adverse event profiles.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Panel: Research in the context

Evidence before this study

We have performed a systematic search on Pub Med and on http://clinicaltrials.gov for randomised clinical trials published in English up to May 1, 2015, evaluating a CMV vaccine in recipients of HCT. Additionally, we searched the abstracts of major oncology and herpes virus conferences. We identified one phase 2 and one phase 3 trial in CMV seropositive HCT recipients. Both trials used the same compound, formerly known as TransVax, and recently renamed ASP0113 (Astellas Pharma Inc, Tokyo, Japan). ASP0113 is a bivalent product containing two plasmids which encode CMV gB and pp65, and is formulated with poloxamer CRL1005 and the cationic surfactant benzalkonium chloride. Mechanistically, the presence of gB protein aims at inducing the production of anti-CMV antibodies, while pp65 would be responsible to induce T-cell mediated responses. The randomised, multicenter, placebo-controlled phase 2 clinical trial was conducted in the USA. Time-to-first episode of viremia was longer in subjects receiving ASP0113, and rates of CMV viremia were lower in the ASP0113 group when measured by a central laboratory. However, ASP0113 failed to show significant reduction in CMV viremia requiring antiviral therapy, and significant increases in pp65 and gB immune responses, using protocol specific analyses. ASP0113 is undergoing an international, randomised, double blind, placebo-controlled, phase 3 study aiming at enrolling 500 CMV seropositive subjects. The main objective of the phase 3 study is to determine if 1-year post-allograft overall mortality is positively affected in patients given ASP0113. The safety of ASP0113 in HCT recipients is also being evaluated.

Added value of this study

CMVPepVax is composed of a cytotoxic pp65 CD8 T-cell peptide epitope, a tetanus T-helper cell peptide epitope, and an innate immunity stimulating adjuvant. CMVPepVax achieves control of CMV viremia, without the need for costly and potentially toxic antivirals, in HLA A*0201 CMV seropositive recipients of HCT. Our findings add value to the existing evidence that immunosuppressed HCT recipients may respond to vaccination early post-HCT, when they are at enhanced risk for CMV reactivation. To our knowledge CMVPepVax data provide the first proof of concept for a CMV vaccine in HCT setting to show vaccine induced increase in $pp65_{495-503}$-specific CD8 T cells, protection from CMV reactivation, and reduced usage of antivirals. Our data confirm previous findings suggesting that humoral immunity is not required for CMV viremia control post-HCT. Thus, addition of gB, as in the ASP0113 vaccine is likely unnecessary in the HCT setting, though prior CMV vaccines used in the setting of solid organ transplant have shown a correlation between humoral immunity (anti-gB) and suppression of CMV viremia. CMVPepVax unexpected clinical outcomes of reduced relapse and increased survival in the VA compare to the OA are also to our knowledge the first proof of concept that an immune therapeutic, stimulating both innate and adaptive arms of the immune response can increase relapse free survival, by achieving clinical benefits for a wide spectrum of major post-HCT complications.

Implications of all the available evidence

Developing a protective CMV vaccine, which may also decrease relapse and increase survival will favorably impact HCT clinical practice, and increase HCT curative potential. Clinical outcomes from the CMVPepVax recently opened phase 2 multicenter, placebo controlled trial, combined with those of the ongoing phase 3 ASP0113 clinical trial will provide a definitive characterization of the properties of these promising candidate vaccines for the HCT setting.
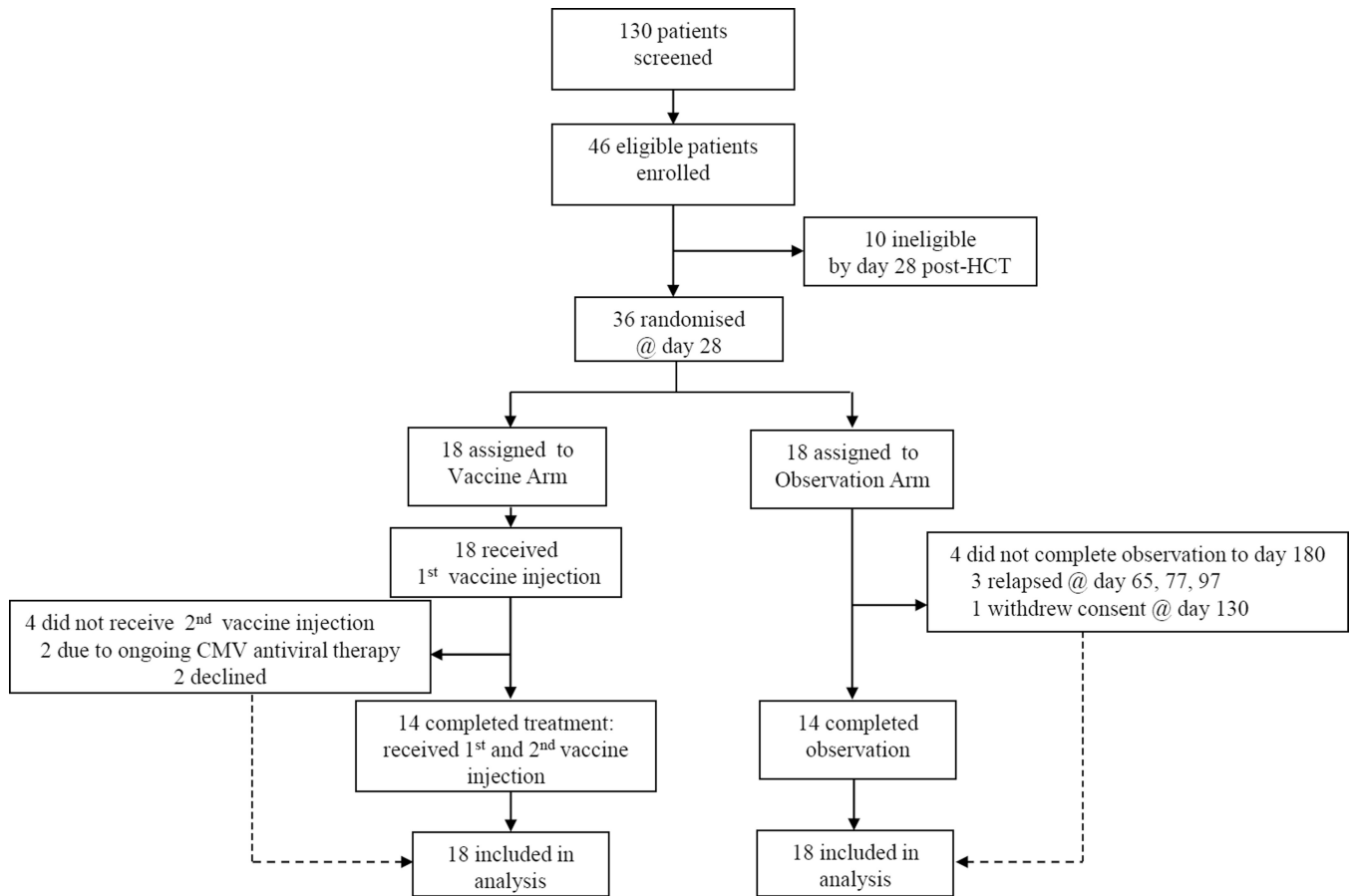
Figure 1. Trial profile
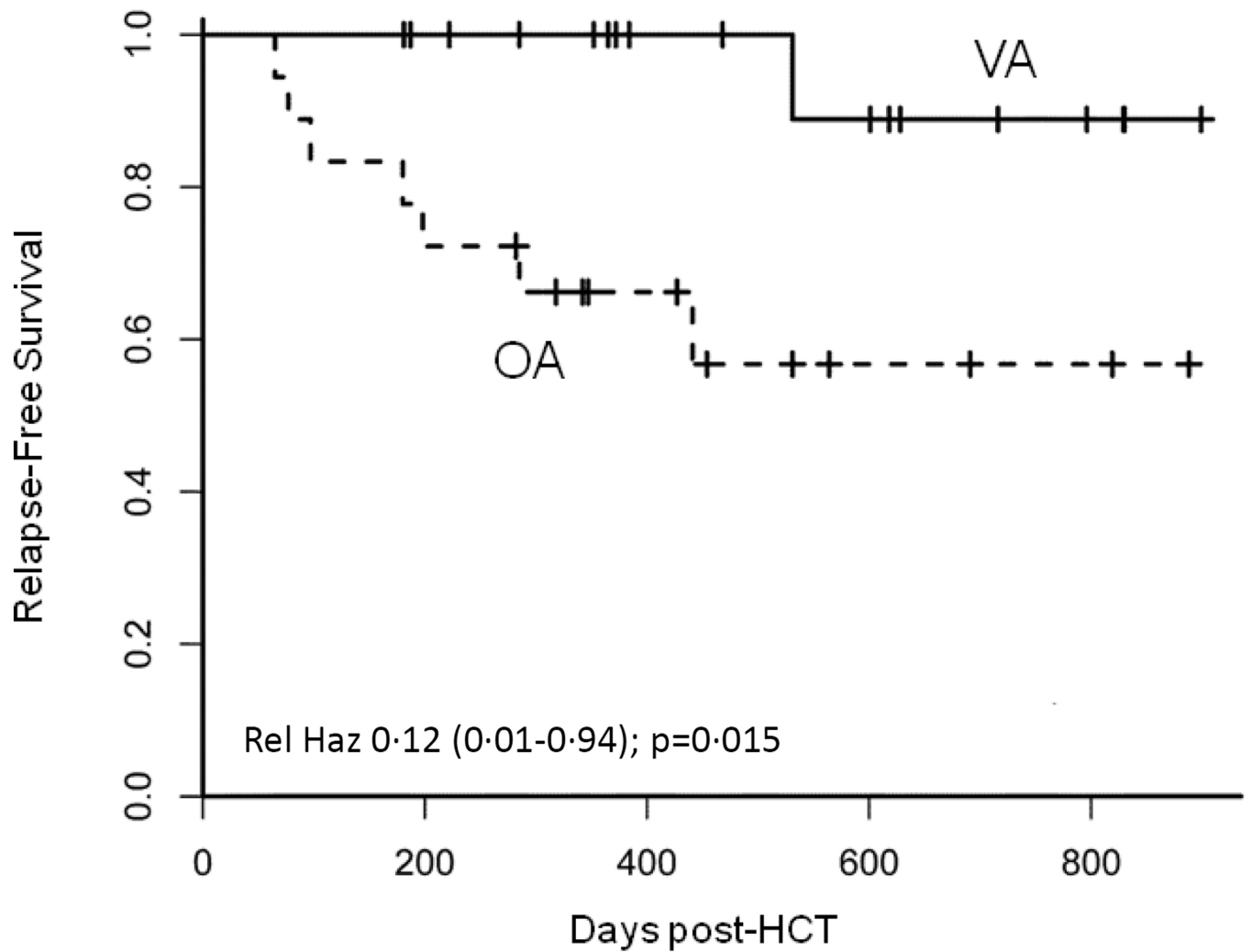
Figure 2. Relapse free survival
Kaplan-Meier estimates of RFS in VA and OA recipients, followed up to May 31, 2015.
Relative hazard (Rel Haz) estimate is shown with 95% CI (0·01–0·94), and 2-sided log-rank
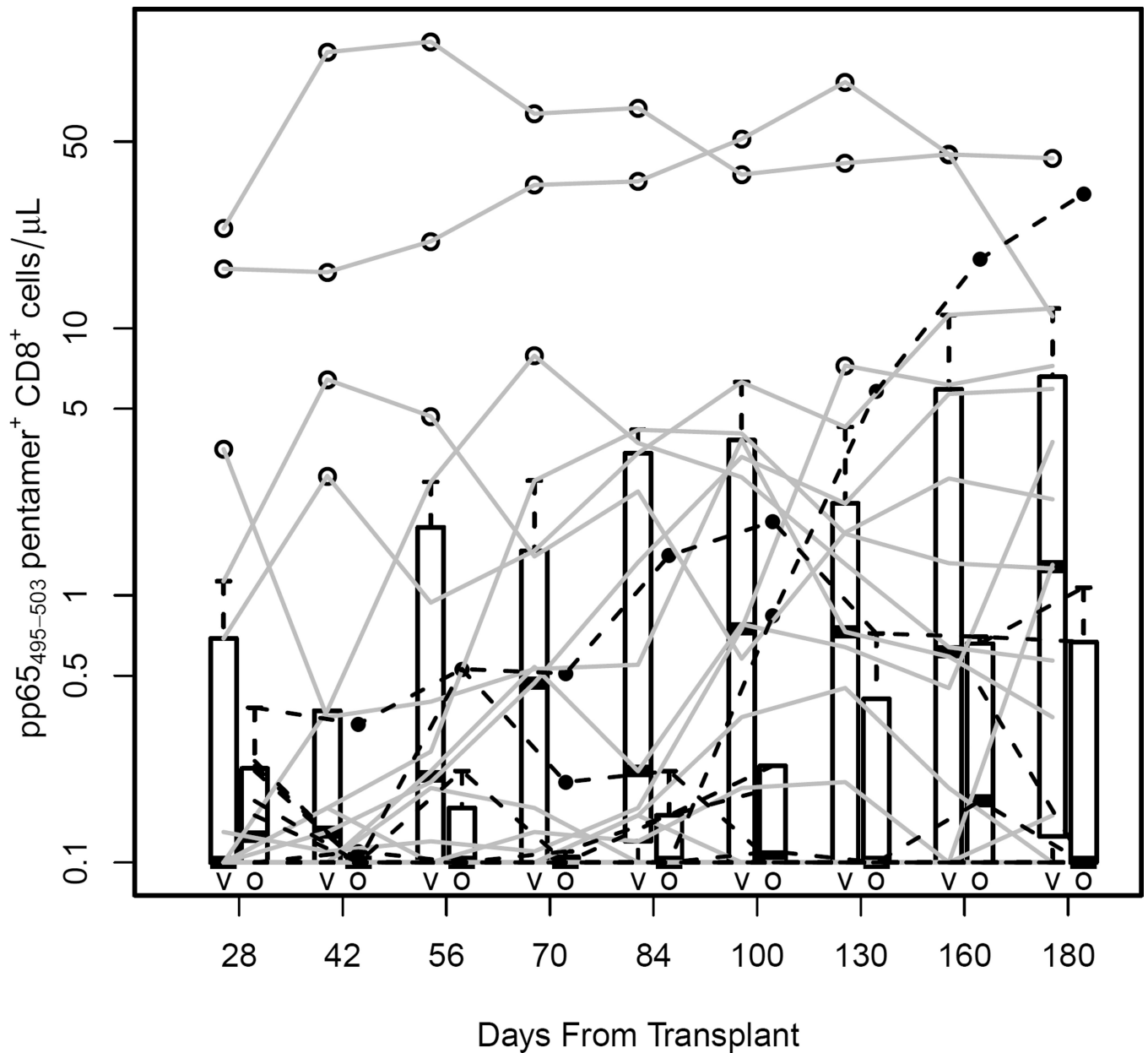p-value. N=number

Figure 3. Levels of pp65$_{495-503}$-specific CD8 T cells
Individual time courses of pp65$_{495-503}$-specific CD8 T cell levels are shown with solid lines for VA patients, and dashed lines for OA patients. Pairs of boxplots show pp65$_{495-503}$-specific CD8 T cell/μl in the VA (v) and OA (o), assessed using pentamer technology, and analysed by FACS Central bars show median; boxes cover central 50% of observations; whiskers extend to at most 1.5 times box length. CMVPepVax was administered on days 28 and 56 to the VA patients. All recipients who did not have CMV reactivation (17 VA patients, and 12 OA patients) were included in this plot.
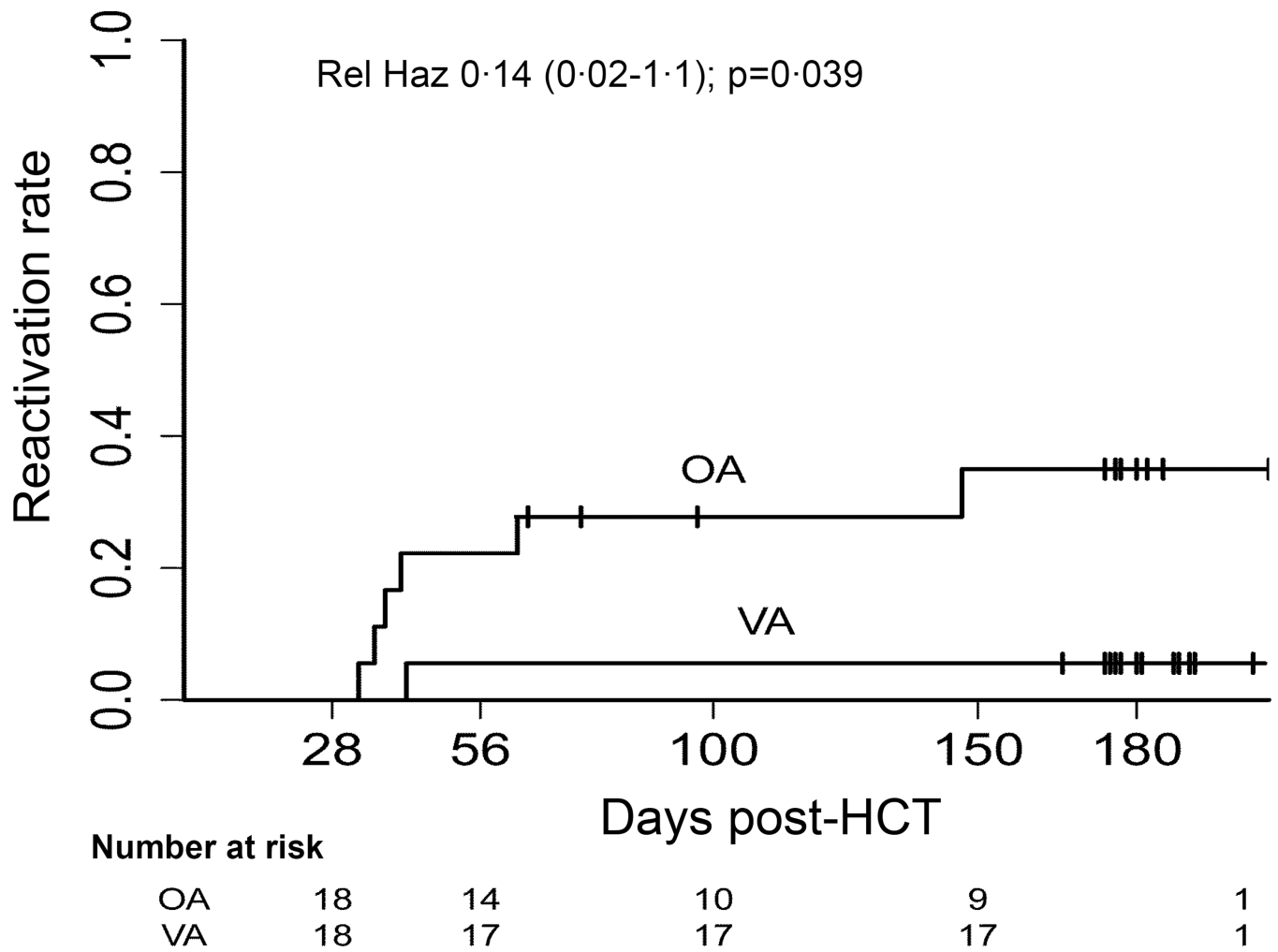
Figure 4. CMV reactivation rate

Kaplan-Meier estimates of CMV reactivation rate in VA and OA recipients, followed for at least 6 months post-HCT. Relative hazard (Rel Haz) estimate is shown with 95% CI (0·02–1·1), and 2-sided log-rank p-value. N=number

Table 1

Baseline characteristics of the 36 hematopoietic stem-cell transplant recipients who were randomised into the study

	Vaccine Arm (n=18)	Observation Arm (n=18)
Sex		
Male	8 (44%)	8 (44%)
Female	10 (56%)	10 (56%)
Age (years)	48·5 (20–72)	56 (20–67)
Donor Type		
Matched Related	7 (39%)	12 (67%)
Matched Unrelated	11 (61%)	6 (33%)
Karnofsky performance score (day 28 post-HCT)	82·2 (6·5, 70–90)	78·9 (5·8, 70–90)
Donor/Recipient CMV status		
+/+	9 (50%)	10 (61%)
−/+	9 (50%)	8 (39%)
Graft Source		
Peripehral Blood Stem Cells	18 (100%)	18 (100%)
Bone Marrow	0 (0%)	0 (0%)
Conditioning Regimen		
Fully ablative	7 (39%)	6 (33%)
Reduced Intensity	11 (61%)	12 (67%)
Primary Diagnosis		
Acute myelogenous/lymphoblastic leukemia	14 (78%)	9 (50%)
Non-Hodgkin/Hodgkin lymphoma	2 (11%)	4 (22%)
Myeloproliferative disorders/Myelofibrosis	1(5·5%)	3 (17%)
Myelodysplastic syndrome	1(5·5%)	1(5·5%)
Chronic myelogenous leukemia	0 (0%)	1(5·5%)
Disease Risk Index (DRI)		
Low	0 (0%)	3 (17%)
Intermediate	15 (83%)	12 (67%)
High	3 (17%)	3 (17%)
Data are n (%) or mean (SD, range).		

Table 2

Selected outcomes for safety, CMV-specific and clinical endpoints in all randomised patients (n=36) followed up to at least 180 days after HCT or until May 31, 2015

Serious Adverse Events	Vaccine Arm (n=18)	Observation Arm (n=18)	p value[a]
Number of patients	4 (22%)	9 (50%)	0·16[b]
Related to CMVPepVax	1	NA	
Grade 3–4 Adverse Events	Vaccine Arm (n=18)	Observation Arm (n=18)	
Number of grade 3–4 Adverse Events	54	91	
	Vaccine Arm (n=18)	Observation Arm (n=18)	p value[a]
Acute GVHD after day 28 post-HCT			0·74[c]
grade I	1 (5·5%)	1 (5·5%)	
grade II	6 (33%)	5 (28%)	
grade III–IV	0 (0%)	0 (0%)	
Disease Relapse [d]	1 (5·5%)	5 (28%)	
Death [d]	0	7 (39%)	
	Vaccine Arm (n=18)	Observation Arm (n=18)	p value[e]
CMV Viremia (500 gc/ml)	1 (5·5%)	6 (33%)	0·044[b]
CMV Disease (gastrointestinal)	1 (5·5%)	1 (5·5%)	0·76[b]
Duration of Pre-emptive CMV Therapy[f]	15 days	263 days	0·015[c]

Data are n (%)

[a]: Two-sided test;

[b]: Fisher's exact test;

[c]: Rank-sum test;

[d]: See Figure 2 for RFS p value;

[e]: One-sided test;

[f]: 500gc/ml CMV viremia