A Phase I Trial of Epstein-Barr Virus Gp350 Vaccine for Children With Chronic Kidney Disease Awaiting Transplantation

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Background. Vaccination against Epstein-Barr virus (EBV), inducing an antibody response to the envelope glycoprotein gp350, might protect EBV-negative children with chronic kidney disease from lymphoproliferative disease after transplantation.

Methods. A phase I trial recruited children with chronic kidney disease to two successive cohorts given three injections of 12.5 μg (n=6) and 25 μg (n=10) recombinant gp350/alhydrogel vaccine over 6 to 8 weeks.

Results. One in each cohort acquired wild EBV before the week 28 evaluation. Both doses were similarly immunogenic, inducing an IgG response in all 13 evaluable patients. Neutralizing antibodies were detected in four recipients (1/4 in the 12.5 μg and 3/9 in the 25 μg cohort). Median time from first vaccination to transplantation was 24 weeks. Immune responses declined rapidly and were unlikely to affect posttransplant events.

Discussion. The vaccine was immunogenic but prolonged effect of vaccination to time of transplantation, or improved adjuvants are required in future trials to reduce posttransplant EBV load and risk of lymphoproliferative disease.

Keywords: Post transplant lymphoproliferative disease, Epstein-Barr Virus, Vaccination.

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Epstein-Barr virus (EBV) contributes to posttransplant lymphoproliferative disease (PTLD) in which impaired EBV-specific T-cell surveillance permits proliferation of virus-transformed B cells. PTLD affects approximately 0.25% of patients with chronic kidney disease (CKD) stage 5 during the first year post renal-transplant when immune suppressive therapy is most intense (*1–3*). Disease risk is higher in EBV-naive patients, mainly children, who acquire the virus in the peritransplant period either by the natural oral route or by virions released from donor-origin B cells.

Antibody responses mainly target intracellular viral capsid (VCA) and Epstein-Barr virus nuclear antigens (EBNA). Their detection marks acute infection and virus carrier-status (*4*). Neutralizing antibodies target EBV envelope glycoprotein gp350. Inducing neutralizing antibodies in EBV-negative individuals awaiting organ grafts might reduce posttransplant viral load acquired orally or from engrafted B-cell passengers. This might also interrupt any amplification of the EBV-transformed population occurring through EBV replicative cycles within PTLD (*5–8*). In a primate model, a gp350-based vaccine pre-

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vented the appearance of EBV-positive B-cell tumors resembling PTLD (*9*) that could be induced by intraperitoneal virus challenge (*10*). In a recent trial in young adults, a gp350 vaccine did not prevent virus acquisition but reduced the incidence of symptomatic infectious mononucleosis, which might imply reduction in viral load (*11*).

We report a phase I trial in children with CKD awaiting transplant to determine an immunogenic safe dose of gp350 plus alum vaccine for phase II trials. Additional off-trial postrenal transplant data on EBV genome levels for vaccinees and nonvaccinees inform the design of future phase II vaccine trials in this patient group.

MATERIALS AND METHODS

The Cancer Research UK Formulation Unit prepared the gp350/0.2% alhydrogel vaccine using a recombinant gp350 protein from a Chinese hamster ovary cell line grown in serum-free medium (*12*). This was buffered to pH 7.2 with phosphate buffer and stored at 2°C to 8°C. PH1/084 was an open, fixed dose escalation, two-center phase I trial sponsored by Cancer Research UK of 12.5 μg and 25 μg gp350 vaccination. A preplanned 50 μg dose level was abandoned, because two batches of the 50 μg dose failed mouse potency tests, and so could not be released for clinical use. The trial and use of additional off-trial anonymous data were approved by the North Somerset and South Bristol Research Ethics Committee. Trial participation was with informed parental consent.

Patients were EBV-negative children with CKD awaiting renal donation without other serious disease, recent surgery, or previous vaccination reactions. Three 3- or 4-weekly subcutaneous vaccinations were given. A fourth vaccination was offered at weeks 30 to 32 for children who were not transplanted, remained negative for EBV infection, and who had a total anti-gp350 antibody level at weeks 26 to 28 below a target level of 300 units of reference standard. This threshold of 300 reference units was selected based on data from 60 EBV seropositive healthy donor samples used to validate the trial assay. Safety was defined as an absence of injection site reaction (ISR; >Common Toxicity Criteria grade 2) or systemic reaction (>grade 1) in all patients who had received at least one vaccination. Patients who received at least one vaccination and who received an organ transplant within 6 months of their final study vaccination were followed up for 12 months posttransplant. Clinical events possibly relating to EBV infection were recorded in these patients.

Wild EBV infection was sought by serology and polymerase chain reaction (PCR) at screening, weeks 7 to 10 (PCR only), weeks 26 to 28, and at the study end. Serum IgG antibodies to EBNA1 and EBV BFRF3-encoded VCA p18 were measured by ELISA, and antibodies to other nonvaccine EBV antigens were measured by immunoblot analysis (*4*, *13*, *14*).
AQ: 2 EBV DNA was measured by a standardized, competitive PCR and LightCycler PCR both targeting a conserved region of the single copy BKRF1 gene encoding EBNA1 using DNA from unfractionated blood (*15–18*). When EBV serology indicated infection, a more sensitive but purely qualitative BamHI-W PCR was used to detect the presence of extremely low levels of EBV DNA (*19*).

Total gp350 antibodies were measured by ELISA (threshold of detection 30 reference units at 1:20 dilution) and neutralizing antibodies (threshold 1000 reference units) by inhibition ELISA using previously published methods (*20*). Evaluable patients had to complete the weeks 7 to 10 measurements after at least 2 vaccinations.

At Great Ormond Street Hospital, an off-trial peritransplant test for anti-EBV VCA IgG and IgM was undertaken, and posttransplant circulating EBV levels were measured using previously described methods (*21*).

RESULTS

Twenty two patients were approached to participate. Six were not eligible, because they were EBV seropositive at screening (n=3) or they proceeded rapidly to transplant (n=3). Sixteen patients (median age 8.9 years, range 1.4–17.6 years) in two centers were vaccinated in sequential 12.5 μg (n=6) and 25 μg (n=10) cohorts (Fig. 1). Two received only two cycles. Patient 2.1 (12.5 μg cohort) underwent transplantation 23 days from first vaccination and thus did not complete the week 7 to 10 evaluation for immunogenicity. Patient 2.3 (25 μg cohort) withdrew from the third vaccination after a grade 2 ISR but completed evaluation until transplantation in week 9. Three patients (25 μg) received a fourth vaccination in week 30 to 32 having met the protocol-specified criteria that the total anti-gp350 IgG was less than 300 units, and they remained EBV-negative by both PCR and serology on blood samples week 26 to 28. F1

Tolerability was good with 6 patients experiencing a total of 8 ISR events (12.5 μg, 1/6 patients experienced grade 1 reactions; 25 μg, 4/10 grade 1, 1/10 grade 2). Only two systemic reactions occurred (25 μg cohort, fever grade 1). The median time from start of vaccination to transplantation was 24 weeks (n=14, range 3–138 weeks).

Four patients acquired wild-type EBV infection during the active on-trial evaluations. Seroconversion was detected by positive results on either or both the ELISA and immunoblot assays for responses to non-gp350 antigens. No symp-

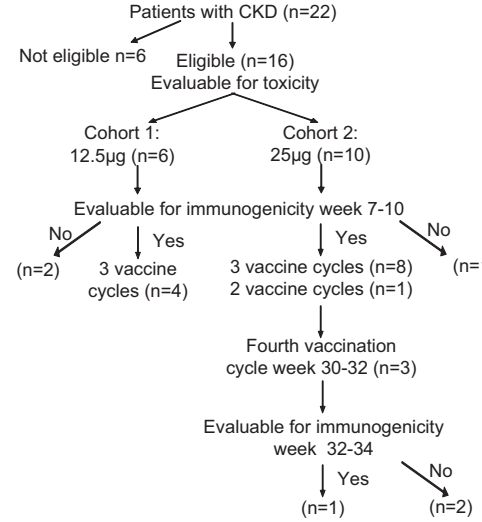

FIGURE 1. Trial structure. The reasons for noneligibility were early transplant or Epstein-Barr virus (EBV) positivity at screening. The reasons for nonevaluability for immunogenicity were early transplant or seroconversion to wild EBV infection. These are described in the text.

toms associated with EBV seroconversion were reported. For two patients, seroconversion was first noted in the sample from week 26 to 28. This was the first sample taken for serology postvaccination, and so the onset of EBV primary infection was not defined. Therefore, these patients were not evaluable for anti-gp350 responses on trial. For two patients, EBV serology was negative at week 26 to 28 but positive at week 32 to 34, after the fourth vaccination. Therefore these patients were evaluable for anti-gp350 IgG response during the first three but not after the fourth vaccinations.

In one of the two study centers, off-trial EBV genome measurements were undertaken routinely from the time of renal transplant. Results were available for eight vaccinees and 31 consecutive pediatric nonvaccinees. The peak EBV genome levels of vaccinees during the first 26 weeks posttransplant were similar to those observed for nonvaccinees (Fig. 2A). Peak posttransplant EBV genome levels were typically higher for children who had not been exposed to EBV before transplant (median 85139, quartiles 22898, 252014) than for the EBV-positive transplant recipients (median 6424, quartiles 357, 18732).

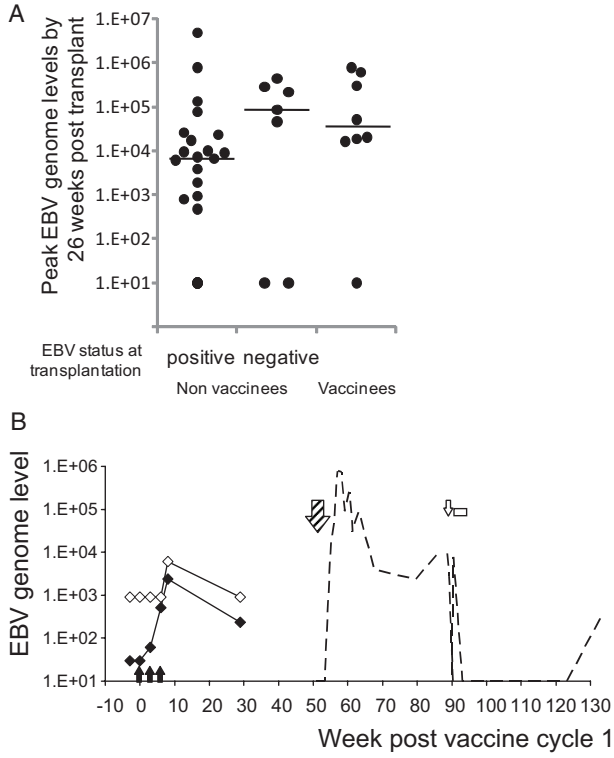

FIGURE 2. Epstein-Barr virus (EBV)-related events posttransplantation in relation to vaccination. (A) Peak EBV circulating load (genomes per mL whole blood) measured 2 weekly up to 26 weeks posttransplant by quantitative polymerase chain reaction in one center for 24 EBV-positive nonvaccinees and 7 EBV-negative nonvaccinees and 8 vaccinees (*solid bar* median). EBV status was determined pretransplant by measuring circulating anti-EBV viral capsid IgG. (B) Relationship between gp350 vaccination (*solid arrows*), total anti-gp350 IgG response (standardized units in ELISA—*solid diamonds*), neutralizing anti-gp350 response (competitive ELISA—*open diamonds*), transplantation (*hatched arrow*), and circulating EBV load (genomes per mL whole blood—*broken line*) for patient 1.5. Posttransplant lymphoproliferative disease was proven on lymph node biopsy (*open arrow*). He was treated with four rituximab infusions (*open rectangle*).

One episode of EBV-related PTLD was reported. Patient 1.05 (12.5 μg cohort) received a live EBV-positive sibling graft. The patient was treated for acute rejection. A high EBV load was reported soon after transplantation. PTLD developed as cervical lymphadenopathy and exudative tonsillitis between 30 and 40 weeks posttransplant. Cervical lymph node biopsy demonstrated predominantly $CD20^+$ pleotropic EBV-positive B-cell blasts with scattered Hodgkin-like cells and admixed $CD3^+$ T cells. This was consistent with immunoblastic monomorphic PTLD or a florid atypical primary EBV infection. The patient was treated with four rituximab infusions with rapid resolution of signs and EBV viremia (Fig. 2B). In the only other EBV-related clinical event after transplantation, patient 1.01 (12.5 μg cohort) was admitted to hospital in response to an asymptomatic peak in measured circulating EBV genomes during 2-weekly surveillance. Azathioprine was stopped. EBV levels fell and did not reach this level again.

All evaluable patients in the 12.5 μg (n=4, Fig. 3A) and 25 μg cohort (n=9, Fig. 3B) made a detectable response to three vaccination cycles (or two cycles for one patient in the 25 μg cohort). The median peak anti-gp350 level was 607 units (range 95–2353) for the 12.5 μg cohort and 612 units (range 66–2087) for the 25 μg cohort. Only four evaluable patients made a neutralizing anti-gp350 antibody response (Fig. 3C): 1/4 in the 12.5 μg cohort and 3/9 in the 25 μg cohort. Three of the four responders with detectable neutralizing antibodies also had the highest peak anti-gp350 response to three vaccine cycles (Fig. 3D). For the only evaluable patient (25 μg, Fig. 3E), the fourth vaccine cycle was followed by a rapid and substantial amplification of anti-gp350 IgG and, for the first time, detection of neutralizing antibodies by ELISA.

DISCUSSION

Highest risk of PTLD for pediatric solid organ graft recipients occurs in the first year after transplantation, when viral transmission may coincide with maximal immune suppression. The overall purpose of pretransplant gp350 vaccination for EBV-naive children with CKD is to decrease the occurrence and severity of PTLD by stimulating protective EBV-specific IgG responses before transplantation. The hypothesis underlying this strategy is, first, that gp350-specific immunoglobulin responses can reduce the burden of EBV-infected cells after primary viral acquisition, and second, that fewer EBV-positive cells results in reduced risk of PTLD.

This phase I trial was not designed to test the effect of vaccination on EBV infection. There was no expectation that gp350 vaccination would prevent acquisition of wild EBV. Interestingly, four children did acquire EBV naturally during the on-trial evaluations as evidenced by seroconversion to antigens other than gp350. There are no data that might suggest that this is an unusually high or low rate of wild EBV infection in this population. A single case of PTLD was noted in the trial cohort. This was a limited stage lymphoproliferation, consistent on histologic examination with either an immunoblastic monomorphic PTLD or a florid atypical response to primary EBV infection and that resolved rapidly to CD20-

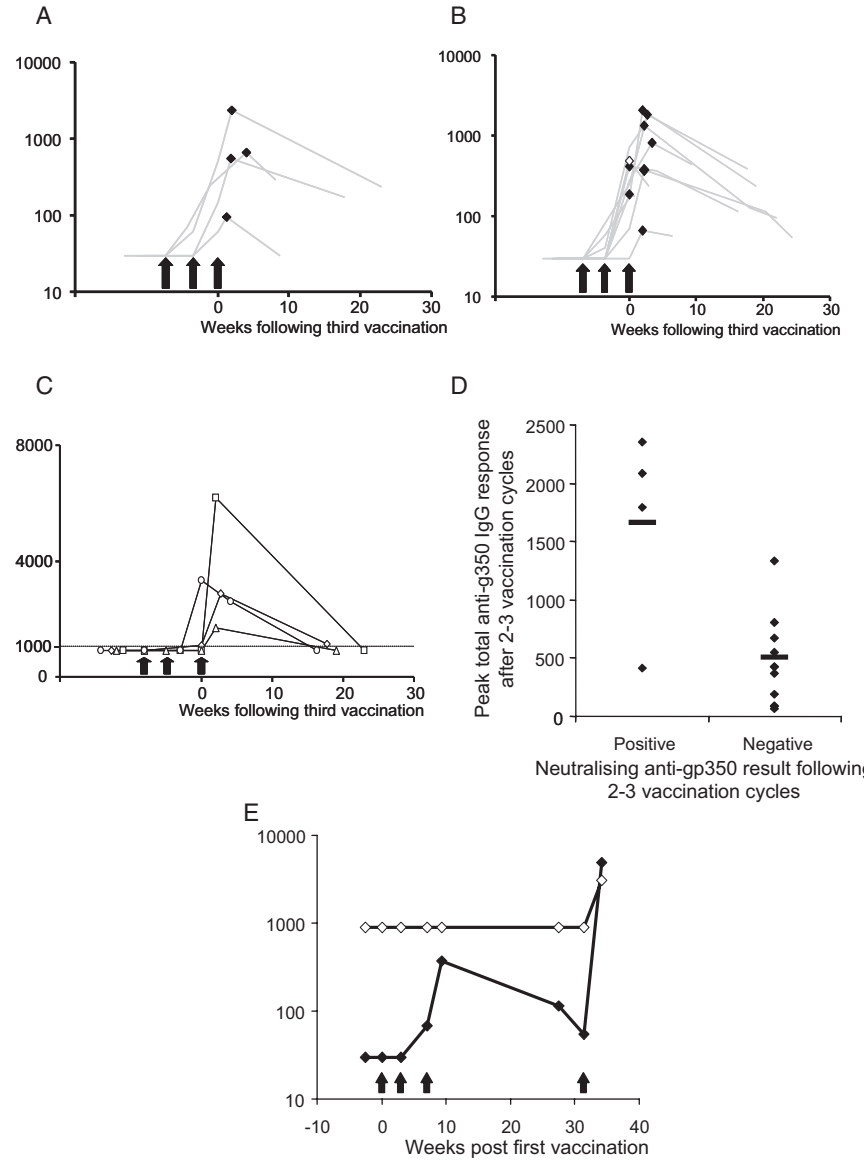

FIGURE 3. Immune response to Epstein-Barr virus (EBV) gp350 vaccination. (A and B) Immune response to three cycles of EBV gp350 vaccination given at 12.5 μg, (A), n=4, and 25 μg, (B), n=9. Peak values (*diamonds*) and kinetics of response (*gray lines*) for total anti-gp350 IgG standardized units measured by ELISA. An anti-gp350 level less than 30 units is taken as the baseline for this assay. Results are presented in relation to each vaccination cycle at 3- to 4-week intervals (*solid arrows*). One individual received only two vaccine cycles (*open diamond*). (C) Anti-gp350 neutralizing antibody responses measured by competitive ELISA. One thousand units is taken as the baseline for this assay. Results are presented for each of four patients (*open symbols*). Results are presented in relation to each vaccination cycle at 3- to 4-week intervals (*solid arrows*). (D) The detection of gp350 neutralizing antibody responses associates with higher total anti-gp350 antibody responses. The peak anti-gp350 IgG response to three vaccine cycles for all patients is shown (*closed symbols*), divided by whether a neutralizing antibody response was detected for each patient. The mean peak response for each group is shown by a *bar*. (E) Kinetics of the immune response of patient 2.9 to four vaccination cycles (*solid arrows*) for total anti-gp350 IgG (*closed diamonds*) and neutralizing antibodies (*open diamonds*).

targeted therapy (Fig. 2B). In the one center in which EBV genome levels were routinely measured at 2-week intervals for all graft recipients, peak measurements in the 26 weeks posttransplant for vaccinees fell within the same range as for a larger cohort of nonvaccinees (Fig. 2A). In summary, these data do not suggest that this three vaccine cycle strategy influenced EBV-related events before or after transplantation.

The purpose of this phase I trial was to test the tolerability and immunogenicity of two dose levels of a gp350 vaccine in this specific patient group, that is, children with CKD awaiting renal transplant. This has demonstrated that both doses were well tolerated. The trial measurements were selected on the assumption that a higher absolute value of anti-gp350 IgG and the detection of neutralizing responses are biomarkers for a protective effect against EBV infection. All evaluable patients made IgG responses and both dose levels appeared similarly immunogenic. The data suggested an association between higher IgG levels and the presence of neutralizing responses. Vaccination was completed within 10 weeks, whereas half the patients underwent transplantation more than 24 weeks from start of vaccination. Total (Fig. 3A and B) and neutralizing (Fig. 3C) anti-gp350 responses declined rapidly after completion of the three vaccine cycles, whereas, in the single evaluable patient, boosting resulted in further immune amplification. Of note, the patient who developed PTLD was transplanted 50 weeks after vaccination started, and anti-gp350 measurements were already declining by week 30 (Fig. 2B).

In summary, although the vaccine itself is immunogenic, the schedule tested in this phase I trial is unlikely to have influenced posttransplant EBV load or prevented PTLD. If this gp350/alum vaccine formulation is tested in a phase II trial, vaccination cycles should be continued up to the time of transplantation. Adjuvants more effective than alum have now been used in human adults (*22*), including in the context of gp350 vaccination to prevent infectious mononucleosis (*11*). The use of newer adjuvants with gp350 vaccination might result in stronger and more durable EBV-neutralizing

antibody responses in children with CKD. A future phase II trial should demonstrate proof of principle that pretransplant vaccination for EBV-naïve children with CKD results in a reduced posttransplant EBV burden. We observed in off-trial data that the peak EBV levels posttransplant for patients EBV-naïve at the time of transplant were typically higher than those who were already EBV carriers (Fig. 2B). These data might be used to define a target range for posttransplant peak EBV genome levels that would demonstrate effective gp350 vaccination in EBV-naïve transplant recipients in a phase II trial.

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