

## ORIGINAL ARTICLE

# Safety of vaccinating sibling donors with live-attenuated varicella zoster vaccine before hematopoietic stem cell transplantation

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Reactivation of varicella zoster virus (VZV), clinically manifested as herpes zoster (HZ) is a common complication after hematopoietic stem cell transplantation (HSCT). The optimum prophylaxis for this disease has not been defined. In this study, we examined the effects of vaccinating donors with a live-attenuated vaccine with particular reference to their immune responses and the outcome of HSCT patients. Forty prospective HLA-matched sibling donors were vaccinated before HSCT. There were humoral immune responses in both seropositive ( $P < 0.01$ ) and seronegative ( $P = 0.058$ ) donors. Cellular immune response was assayed in 26 donors. Significant correlation was observed between cellular immune responses as enumerated by thymidine incorporation and interferon  $\gamma$  secretion ( $P < 0.001$ ) and the latter was used in subsequent analyses. Significant response was observed in seronegative (6/26) and a group of seropositive (13/26) donors while 7/26 seropositive donors showed no response. Thirty-four HSCT were performed. These patients have a lower, albeit insignificant, risk of HZ compared with historical controls and only 3/34 patients developed single dermatomal HZ at 6, 9 and 28 months after HSCT. No patients developed VZV-related mortality. Vaccinating donors with live-attenuated VZV vaccine was safe, but whether it confers a significant protection to the patients would require further study.

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## Introduction

Varicella zoster virus (VZV) reactivation, clinically manifested as herpes zoster (HZ), is a major complication after hematopoietic stem cell transplantation (HSCT), affecting up to 50% of patients.<sup>1,2</sup> Despite treatment with high-dose acyclovir, most patients suffer from its complications including post-herpetic neuralgia, corneal ulceration, viral dissemination and secondary bacterial infection. The pathogenesis of VZV reactivation is not completely understood, but cell-mediated immunity is believed to play an important role in the host response to VZV reactivation.<sup>3</sup>

At present, there is no effective prophylaxis against VZV reactivation, and HZ continues to be a significant cause of morbidity among HSCT patients. Continuous acyclovir prophylaxis post-HSCT may reduce the risk of HZ,<sup>4</sup> but HZ commonly occurs after acyclovir is discontinued. Vaccination of recipients with heat-inactivated VZV vaccine may also reduce the risk of HZ, but repeated doses are needed to sustain the host immunity.<sup>5</sup>

Live-attenuated VZV vaccine, which is commonly included in the vaccination program for children,<sup>6</sup> is not widely used in HSCT patients because of the concern of vaccine virus infection. On the other hand, we and others have shown that adoptive transfer of immunity may play a role in the clearance of latent hepatitis B virus<sup>7</sup> and Epstein–Barr virus after HSCT.<sup>8</sup> This has led us to hypothesize that boosting donor VZV immunity pre-HSCT might prevent VZV reactivation in HSCT recipients. In this prospective study, we vaccinated HSCT donors with a live-attenuated VZV vaccine before transplantation. The primary end points were the immunological responses in the donors and the safety to the patients. The secondary end points were the occurrence of HZ in these patients.

## Materials and methods

### *Patients and HSCT protocols*

Forty patients and their HLA-matched sibling donors who underwent allogeneic HSCT from January 2002 to December 2005 were recruited. Patients who underwent allogeneic HSCT from HLA-identical sibling donors over the same

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period of time were retrospectively recruited as historical controls. Anti-microbial and prophylaxis against graft-versus-host disease (GVHD) during HSCT have been described previously.<sup>9</sup> Briefly, all patients were given acyclovir (5 mg/kg every 8 h) for prophylaxis against herpes simplex infection, from the commencement of conditioning to day 30 after BMT. Anti-bacterial and anti-fungal prophylaxes were also administered over the same period of time. Prophylaxis against GVHD comprised methotrexate (15 mg/m<sup>2</sup> on day 1 and 10 mg/m<sup>2</sup> on days 3, 6 and 11) and cyclosporine (3 mg/kg intravenously or 8 mg/kg orally days 1–50 tailed off at 6 months). Patients developing GVHD received additional immunosuppression at the discretion of the attending physician.

#### *VZV vaccination*

Donors were vaccinated with either one or two doses of a live-attenuated VZV vaccine of the Oka strain (Varilrix SmithKline Beecham, Belgium) at 8 and/or 4 weeks before HSCT (0.5 ml, subcutaneously), depending on the timing of HSCT, which was determined by the recipients' underlying condition. The investigation was approved by the institutional review board in accordance with the Declaration of Helsinki.

#### *Anti-VZV antibody assay*

Anti-varicella IgG was determined by enzyme-linked fluorescent immunoassay based on the VIDAS VZG assay (VZG; bioMerieux/Vitek, Marcy l'Etoile, France) as per the manufacturer's instructions. Purified VZV antigen was immobilized on a plastic support and incubated with patients' serum. Specific anti-VZV IgG present in the serum was detected by mouse monoclonal anti-human IgG conjugated with alkaline phosphatase. A fluorescent substrate (4-methylumbelliferone) was then added and hydrolyzed into a fluorescent product detected by spectrometer at 450 nm. The fluorescent intensity was proportional to the quantity of anti-VZV IgG. Sero-positivity was defined by an arbitrary value of >0.9 and sero-negativity ≤0.9.

#### *Cellular immunity assay by IFN- $\gamma$ release and [<sup>3</sup>H]thymidine incorporation*

Mononuclear cells were isolated from peripheral blood by density-gradient centrifugation (Ficoll-Paque Plus, Amersham Biosciences, Uppsala, Sweden) and stored in liquid nitrogen until use. During assay, the cells were thawed and incubated in 96-well round-bottom microtiter plates in Rosewell Park Memorial Institute 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with autologous serum (10%), penicillin (0.1 U/l)–streptomycin (0.1 g/l) (Invitrogen, CA, USA) and sodium pyruvate (0.11 g/l) (Invitrogen, CA, USA). Responder cell frequency (RCF) was determined by limiting dilution method from 24 replicate cultures at 50 000, 25 000, 12 500, 6250 and 3125 PBMC per well. These were cultured either with VZV antigen (Dade Behring, Eschborn, Germany) or a negative control CMV antigen (a herpesvirus with distinct antigenicity) for 10 days.<sup>10</sup> Phytohemagglutinin and concanavalin A (both from Sigma, St Louis, MO, USA) were also added to control wells on day 7 to test the viability of the PBMC in

the test. Supernatant of each well (50  $\mu$ l) was harvested and tested on day 10 for interferon- $\gamma$  (IFN- $\gamma$ ) by a human IFN- $\gamma$  ELISA kit (eBioscience, San Diego, CA, USA). The remaining cells in culture were then pulsed for at least 8 h with 0.25 mCi of [<sup>3</sup>H]thymidine (Amersham, Bucks, UK) per well. Responder wells were defined as those with greater than the mean counts per minute + 3 s.d. of the 24 replicate parallel control cultures, and RCF was calculated according to Poisson's statistics.<sup>11</sup>

#### *Statistical analysis*

Comparison between groups of data was evaluated by Mann–Whitney *U*-test. Cox regression analysis was used to evaluate the risk of HZ. Both donor and patient VZV serostatus pre-HSCT, conditioning regimens and occurrence of acute and chronic GVHD were entered as competing risks (SPSS, Chicago, IL, USA). *P*-value less than 0.05 was considered statistically significant.

## **Results**

#### *Patient characteristics*

A total of 40 sibling donors were vaccinated from whom 34 HSCT were successfully performed. Six donors who were vaccinated did not donate because the patients' condition became unsuitable for transplantation according to the physicians' discretion. The clinical characteristics of the transplanted patients are shown in Table 1. There was no difference between patients whose donors have been vaccinated and historical controls (whose donors were not vaccinated) in patient demographics, underlying diseases, source of HSC, conditioning regimens, patients' and donors' serostatus pre-HSCT and the occurrence of acute GVHD.

#### *Donor humoral response to VZV vaccination*

Humoral response to VZV vaccination was assessed in 40 sibling donors. Both sero-negative ( $n=7$ ) and sero-positive ( $n=33$ ) donors showed an increase in anti-VZV titer upon vaccination at medians of 2.42 (sero-positive) and 2.33 (sero-negative) months post-vaccination (Figure 1).

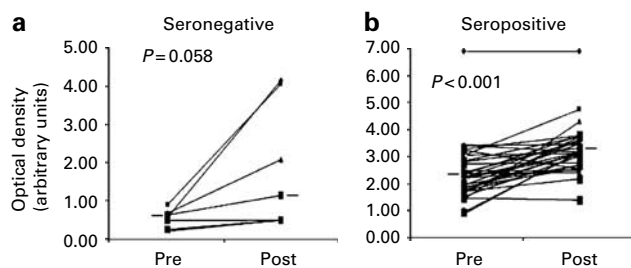
#### *Cellular response to VZV vaccination*

The cellular response to VZV based on both lymphocyte proliferation and IFN- $\gamma$  secretion was measured in 26 HSCT donors. There was significant correlation between these assays ( $P<0.001$ ). However, discordant results were seen in eight assays in whom IFN- $\gamma$  secretion was not accompanied by lymphocyte proliferation (Figure 2a). In subsequent analyses, cellular immunity was defined by IFN- $\gamma$  secretion. Six out of 26 donors were sero-negative and there was a significant increase in cellular immunity to VZV vaccination. Twenty out of 26 donors were sero-positive of whom 13 showed significant increases in cellular immunity (Figure 2b). Seven sero-positive donors who were vaccinated did not show any increase in cellular immunity. There was no significant correlation between humoral and cellular immune responses to vaccinations (data not shown).

**Table 1** Clinicopathologic characteristics of patients

	Donor vaccinated	Donor not vaccinated	P-value
<i>N</i>	34	178	
Age (median, range)	40.5 (19–56)	43.5 (16–65)	0.210
Male/female	18/16	99/79	0.851
<i>Diagnosis</i>			
AML	11	57	0.949
ALL	6	29	
CML	8	34	
Myeloma	4	23	
Others	5	35	
<i>Conditioning regimens</i>			
Bu-Cy	20	95	0.939
Cy-TBI	7	44	
Flu-TBI	5	29	
Others	2	10	
<i>Source of HSC</i>			
BM	26	127	0.677
PBSC	8	51	
<i>Patient</i>			
VZV sero-positive	28	157	0.314
VZV sero-negative	4	18	
Not available	2	3	
<i>Donor</i>			
VZV sero-positive	39	167	0.368
VZV sero-negative	4	7	
Not available	1	4	
<i>aGVHD</i>			
Overall grade $\geq 2$	2	24	0.268
Overall grade $\leq 1$	32	154	
Mortality (non-relapse related) within 12 months	2	39	0.032

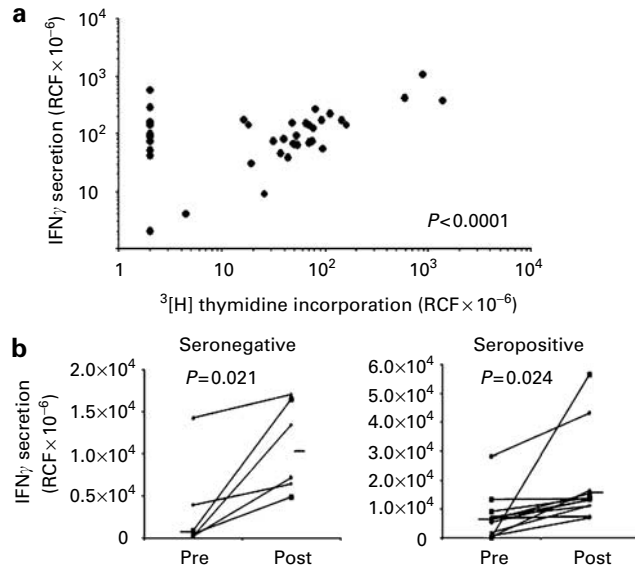
Abbreviations: AML = acute myeloid leukemia; ALL = acute lymphoblastic leukemia; CML = chronic myeloid leukemia; Bu = busulfan; Cy = cyclophosphamide; Flu = fludarabine; TBI = total body irradiation; BM = bone marrow; PBSC = peripheral blood stem cells; aGVHD = acute graft-versus-host disease.



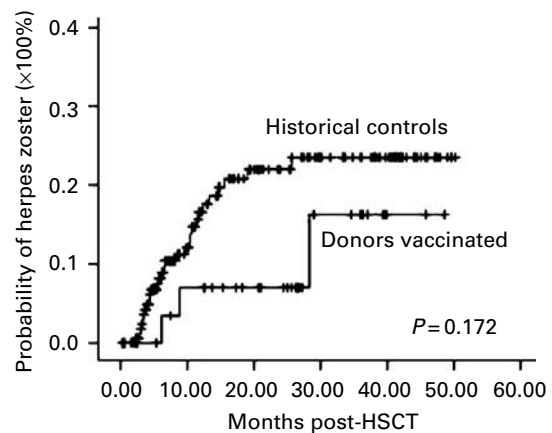
**Figure 1** Donor humoral responses to VZV vaccination. Each dot represents individual data in sero-negative (a) and sero-positive (b) donors and the horizontal bars represent the median in each group.

*Outcome of patients receiving HSCT from vaccinated donors*

Among the 34 patients who received HSCT from VZV vaccinated donors, three developed HZ at 6, 9 and 28



**Figure 2** Donor cellular responses to VZV vaccinations. (a) A significant correlation of cellular immunity as enumerated by IFN- $\gamma$  secretion and  $^3\text{H}$  incorporation. (b) Cellular immune response as enumerated by IFN- $\gamma$  secretion in sero-negative and sero-positive donors.



**Figure 3** Probability of HZ in HSCT patients whose donors were vaccinated as compared with historical control whose donors were not.

months post-transplantation. In all three cases, HZ was cutaneous and confined to a single dermatomal distribution with no dissemination. The donors of these patients were sero-positive before vaccination and they showed significant humoral responses (3.2-, 2.7- and 2.0-fold increase respectively) to vaccination. The two patients who developed HZ at 6 and 9 months received HSCT from donors whose cellular immunity did not respond to VZV vaccination and the patient who developed HZ at 28 months received HSCT from her donor whose immunity showed a 40-fold increase upon vaccination. When compared with historical controls who underwent allogeneic HSCT from HLA-matched sibling donors, patients who received HSCT from VZV vaccinated donors appeared to have a lower, albeit not significant, risk of VZV reactivation after HSCT (odds ratio: 0.436, 95% CI: 0.133–1.434,  $P=0.172$ , Figure 3). None of the patients in the donor-vaccinated

group have developed VZV-related mortality post-HSCT. Intriguingly, patients whose donors were vaccinated had lower risk of non-relapse-related mortality compared with the unvaccinated counterparts ( $P=0.032$ , Table 1).

## Discussion

In the present study, we vaccinated 40 HSCT donors with live-attenuated VZV vaccine and examined the effects on their VZV-specific humoral and cellular immunity and the outcome of their HSCT recipients with special reference to the occurrence of HZ after transplantation. We showed in both sero-negative and sero-positive donors that the vaccine can boost both humoral and cellular immunity against VZV. Intriguingly, among the 26 donors whose cellular immune response was measured, six (23.1%) were sero-negative. Whether this might be attributed to the arbitrary fluorescent intensity cutoff of 0.9 in the fluorescent immunoassay, thereby reducing the sensitivity of the assay, remains to be further evaluated. Notwithstanding this limitation, our results were consistent with previous study in which live-attenuated vaccine was shown to boost VZV-specific immune response in sero-positive people.<sup>12</sup> In addition, our results raise a number of issues that may shed light on a better prophylactic strategy of VZV reactivation post-HSCT.

We demonstrated for the first time the safety of vaccinating HSCT donors with live-attenuated VZV vaccine. To our knowledge, this issue has not been addressed previously. Earlier studies in children with leukemia have raised concern of infection by vaccine virus in susceptible hosts.<sup>13</sup> In the present study, none of the patients developed disseminated VZV infection. Three (out of 34) patients who received HSCT from vaccinated donors developed single dermatomal HZ, representing reactivation of endogenous VZV rather than primary infection by vaccine virus. It was intriguing that patients receiving HSCT from vaccinated donors had lower mortality compared with the unvaccinated counterparts. In fact, the overall survival of patients who received HSCT from vaccinated donors also appeared higher than the historical controls, whose donors were not VZV vaccinated (unpublished observation). These issues would have to be more carefully examined in a future prospective study.

Furthermore, a comparison with historical controls showed that patients who received HSCT from vaccinated donors appeared to have lower, albeit nonsignificant, risk of HZ after transplantation. This observation may be consistent with the notion of adoptive transfer of immunity against VZV from the donor to the patient during transplantation. However, the small number of patients who developed HZ in the vaccinated arm rendered it difficult to ascertain the correlation between donor vaccination response and patients' risk of VZV reactivation. In a few patients in whom adequate serial blood samples were available, we were able to demonstrate an increase in cellular immunity to VZV post-HSCT (unpublished). Nonetheless, spontaneous recovery of VZV immunity is known to occur after transplantation;<sup>14</sup> so the origin of VZV immunity in patients who received HSCT from vaccinated donors would also have to be critically examined.

At present, the optimal prophylaxis for HZ in HSCT patients has not been defined. A randomized controlled study has demonstrated that vaccinating HSCT patients with heat-inactivated VZV vaccine can reduce the risk of HZ.<sup>5</sup> However, the regimen included multiple vaccinations and the benefit was demonstrated only in autologous HSCT patients, whose risk of HZ is generally lower than that of allogeneic HSCT recipients.<sup>2</sup> More recently, a randomized double-blind, placebo-controlled study demonstrated that high-dose acyclovir prophylaxis up to 1 year significantly reduces the risk of HZ among allogeneic HSCT patients.<sup>4</sup> However, HZ may occur after discontinuation of prophylaxis, especially among patients who received continuous immunosuppressive therapy. Therefore, our study may provide ground for further study of the effect of vaccinating donors with live-attenuated VZV vaccine on the occurrence of HZ among allogeneic HSCT patients.

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## References

- 1 Steer CB, Szer J, Sasadeusz J, Matthews JP, Beresford JA, Grigg A. Varicella-zoster infection after allogeneic bone marrow transplantation: incidence, risk factors and prevention with low-dose aciclovir and ganciclovir. *Bone Marrow Transplant* 2000; **25**: 657–664.
- 2 Leung AY, Yuen KY, Cheng VC, Lie AK, Liang R, Kwong YL. Clinical characteristics of and risk factors for herpes zoster after hematopoietic stem cell transplantation. *Haematologica* 2002; **87**: 444–446.
- 3 Arvin AM. Varicella-zoster virus: pathogenesis, immunity, and clinical management in hematopoietic cell transplant recipients. *Biol Blood Marrow Transplant* 2000; **6**: 219–230.
- 4 Boeckh M, Kim HW, Flowers ME, Meyers JD, Bowden RA. Long-term acyclovir for prevention of varicella zoster virus disease after allogeneic hematopoietic cell transplantation – a randomized double-blind placebo-controlled study. *Blood* 2006; **107**: 1800–1805.
- 5 Hata A, Asanuma H, Rinki M, Sharp M, Wong RM, Blume K et al. Use of an inactivated varicella vaccine in recipients of hematopoietic-cell transplants. *N Engl J Med* 2002; **347**: 26–34.
- 6 Vazquez M, LaRussa PS, Gershon AA, Steinberg SP, Freudigman K, Shapiro ED. The effectiveness of the Varicella vaccine in clinical practice. *N Engl J Med* 2001; **344**: 955–960.
- 7 Lau GK, Lok AS, Liang RH, Lai CL, Chiu EK, Lau YL et al. Clearance of hepatitis B surface antigen after bone marrow transplantation: role of adoptive immunity transfer. *Hepatology* 1997; **25**: 1497–1501.
- 8 Bollard CM, Kuehnle I, Leen A, Rooney CM, Heslop HE. Adoptive immunotherapy for posttransplantation viral infections. *Biol Blood Marrow Transplant* 2004; **10**: 143–155.
- 9 Leung AY, Suen CK, Lie AK, Liang RH, Yuen KY, Kwong YL. Quantification of polyoma BK viruria in hemorrhagic cystitis complicating bone marrow transplantation. *Blood* 2001; **98**: 1971–1978.

- 10 Svahn A, Linde A, Thorstensson R, Karlen K, Andersson L, Gaines H. Development and evaluation of a flow-cytometric assay of specific cell-mediated immune response in activated whole blood for the detection of cell-mediated immunity against varicella-zoster virus. *J Immunol Methods* 2003; **277**: 17–25.
- 11 Taswell C. Limiting dilution assays for the determination of immunocompetent cell frequencies. I. Data analysis. *J Immunol* 1981; **126**: 1614–1619.
- 12 Levine MJ, Ellison MC, Zerbe GO, Barber D, Chan C, Stinson D *et al*. Comparison of a live attenuated and an inactivated varicella vaccine to boost the varicella-specific immune response in seropositive people 55 years of age and older. *Vaccine* 2000; **18**: 2915–2920.
- 13 Hardy I, Gershon AA, Steinberg SP, LaRussa P. The incidence of zoster after immunization with live attenuated varicella vaccine. A study in children with leukemia. Varicella Vaccine Collaborative Study Group. *N Engl J Med* 1991; **325**: 1545–1550.
- 14 Meyers JD, Flournoy N, Thomas ED. Cell-mediated immunity to varicella-zoster virus after allogeneic marrow transplant. *J Infect Dis* 1980; **141**: 479–487.