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Long-Term Follow-Up of Antibody Titers Against Measles, Mumps, and Rubella in Recipients of Allogenic Hematopoietic Cell Transplantation

Jan Bögeholz[1,2], Norman F. Russkamp[1], Christian M. Wilk[1,2], Elise Gourri[3], Eugenia Haralambieva[4], Urs Schanz[1], Nicolas J. Mueller[5], Markus G. Manz[1], Antonia M.S. Müller[1,*]

[1] *Department of Medical Oncology and Hematology, University Hospital Zurich, Zurich, Switzerland*
[2] *Department of Internal Medicine, University Hospital Zurich, Zurich, Switzerland*
[3] *Department of Molecular Diagnostics and Research & Development, Blood Transfusion Service Zurich, Swiss Red Cross, Zurich, Switzerland*
[4] *Department of Pathology, University Hospital Zurich, Zurich, Switzerland*
[5] *Department of Infectious Diseases & Hospital Epidemiology, University Hospital Zurich, Zurich, Switzerland*

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Outbreaks of viral infections, such as measles, are regularly observed and pose a serious threat to recipients of allogeneic hematopoietic cell transplantation (HCT). The questions of how long cellular and humoral protective host immunity persists, and whether donor immunity can be transferred has not been clarified. Here we present a retrospective analysis of humoral immunity—serial antibody titers against measles, mumps, and rubella—in 331 patients who underwent allogeneic HCT at our single center between 2002 and 2015. Associations between the loss of protective antibody levels and clinical patient characteristics and transplantation parameters were examined. In general, antibody protection against measles persisted longer, with 72% of patients maintaining sufficient titers at 5 years post-HCT even without revaccination, while at that time only 65% and 50% of patients had protective immunity against rubella and mumps, respectively. The great majority of donors were seropositive for all 3 viruses; however, it appeared that donor humoral immunity could not be transferred and had no impact on post-HCT serostatus. Rather, the most relevant factor for persistent protective antibody titers against measles and rubella was whether patients were born before the introduction of the respective vaccine and thus were immunized by the wild-type disease-inducing virus instead of the vaccine. Moreover, the presence of moderate and severe chronic graft-versus-host disease (GVHD) was associated with more rapid loss of immune protection. In contrast, underlying disease, intensity of the conditioning regimen, use of antithymocyte globulin, age, and graft source had no influence on antibody titers. Overall, our findings suggest that the majority of antibodies against measles, mumps, and rubella originate from residual host cells, whereas donor immune status appears to have no influence on antibody protection post-HCT.

INTRODUCTION

Infections are a major cause of morbidity and mortality in recipients of allogeneic hematopoietic cell transplantation (HCT) [1,2]. Whether immune protection can be transferred from donor to host by adoptive transplantation of mature lymphocytes within an allograft has not been completely clarified [2-7]. Likewise, little is known about the persistence of residual host-type memory B and plasma cells, their ability to provide protective antibodies post-allogeneic HCT, and whether this host-type immunity is influenced by clinical parameters, such as pretransplantation conditioning and pharmacologic immunosuppression.

All possible measures are taken to protect vulnerable patients against infections post-HCT. In addition to antimicrobial prophylaxis against some pathogens, intravenous immunoglobulins can be administered, and reimmunization using inactivated vaccine is initiated within the first year post-HCT [8,9]. However, due to concerns about the safety of attenuated live vaccines [10-13] and limited data on their use in the post-HCT setting, it is recommended that live vaccines not be given within the first 2 years post-HCT, and thereafter given only in the absence of graft-versus-host disease (GVHD) and immunosuppressive medication [8,9,14-17]. Thus, many transplant recipients are not vaccinated against measles, mumps, and rubella (MMR). Exposure to these viruses cannot always be controlled, however, particularly in an era of "vaccine fatigue"

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*Correspondence and reprint requests: Antonia M.S. Müller, Department of Medical Oncology and Hematology, University Hospital Zurich, Rämistrasse 100, CH-8091 Zürich, Switzerland.
E-mail address: antoniamaria.mueller@usz.ch (A.M.S. Müller).

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and antivaccination movements. In fact, many regions have insufficient vaccination coverage against MMR, and increasing numbers of nonimmunized individuals [18,19]. As a consequence, regular outbreaks of much-feared viral infections, particularly measles, occur throughout Europe and the United States [18-21]. The number of measles patients reported to the Swiss authorities from January 1 and May 6, 2019, was almost 8 times higher compared with the same time period the previous year (166 versus 21), including 2 adults who died from measles (1 of who was immunocompromised due to cancer treatment) [22]. Measles can lead to detrimental long-term complications in healthy children. Infections in immunocompromised patients, including transplant recipients, often result in an aggravated clinical course and more short- and long-term complications, and can even be fatal [23-27].

In situations of uncertainty or when a patient has been accidentally exposed, the duration of host protection is relevant. Already in the 1980s and 1990s, it was observed that levels of antibodies against MMR decline over time [28-30]. The implications of newer HCT techniques, such as an increasing use of reduced-intensity conditioning (RIC) regimens, use of mobilized peripheral blood (MPB) instead of bone marrow (BM) grafts, changes in donor source (more matched unrelated donors, fewer matched sibling donors), and the broad application of antithymocyte globulin (ATG) as GVHD prophylaxis, on the course and decline of protective antiviral antibody titers post-allogeneic HCT have not been studied in detail until now.

Here we report our long-term observations in 331 consecutive allogeneic HCT recipients at the University Hospital Zurich regarding antibody titers against MMR during a follow-up period of up to 13 years. Until recently at our center, the majority of patients did not routinely receive the MMR vaccine post-transplantation, even in the absence of GVHD and beyond 2 years post-HCT. Patients who received the MMR vaccine were excluded from our analysis to obtain a homogenous patient cohort in which the natural decline of antibody levels post-transplantation could be assessed. Despite a known half-life of 5 to 21 days for immunoglobulins [31-33], antibody titers are measurable for years, even without revaccination, suggesting that antibodies are derived from surviving residual host cells. Knowledge of persisting humoral host immunity is important in clinical practice to estimate the risks in certain patient cohorts.

METHODS

Patients

We retrospectively identified patients who underwent allogeneic HCT at the University Hospital Zurich between 2002 and 2015. Antibody titers were obtained from each patient before and after HCT during routine clinical visits and laboratory workups. For related donors, antibody titers were assessed before hematopoietic cell donation. Our analysis included only patients who remained in remission beyond 12 months post-HCT, because most of the patients with early relapse did not have long-term follow-up data. Patients who received an MMR vaccination at any time post-HCT were also excluded. A total of 331 patients were analyzed within the study. The study was approved by the local Ethics Commission (Cantonal Ethics Committee Zurich; BASEC-no 2018-01612) and was conducted in accordance with the principles embodied in the Declaration of Helsinki.

Antibody Titers

Antibody titers against common viruses and other pathogens were obtained before HCT, at 6 months post-HCT, and annually thereafter on a routine basis during checkups at our transplantation center. Antibodies against MMR were measured by enzyme-linked immunosorbent assay (ELISA; performed at the Institute for Virology, University of Zurich), cutoff levels were defined as >200 mIU/mL for measles (Serion ELISA *classic* Measles Virus IgG assay; Serion, Wurzburg, Germany), >.5 RFU for mumps (Serion ELISA *classic* Mumps Virus IgG assay), and ≥15 IE/mL for rubella (VIDAS RUB IgG; bioMerieux, Marcy l'Etoile, France). Borderline titers (measles, 100 to 200 mIU/mL; mumps, .3 to .5 RFU; rubella, 10 to 15 IE/mL) were classified as loss of protective immunity against the respective virus.

Conditioning Regimens and GVHD Prophylaxis

Conditioning regimens used were either myeloablative (MAC) or reduced-intensity (RIC). The most commonly used MAC regimen was cyclophosphamide (Cy) 60 mg/kg/day, on days -7 to -6 plus total body irradiation (TBI) 12 to 13.2 Gy, with or without ATG, followed by busulfan (Bu) 4 × 1 mg/kg on day -7, and adjusted to plasma levels on days -6 to -4 and Cy 60 mg/kg/day on days -3 to -2. Other MAC regimens used were Cy/alemtuzumab/TBI, fludarabine (Flu)/Bu 4 with and without ATG, and Vp16/Cy/TBI.

The standard RIC regimen used at our center is Flu/Bu (Flu 30 mg/m^2/day on days -7 to -2 and Bu 4 × 1 mg/kg on the first day and adjusted to plasma levels on days -3 to -2) with and without ATG. Other RIC regimens were Flu/Cy/TBI with and without ATG, Cy/ATG, FLAMSA, Flu/ATG, Flu/ATG/Mel, and Flu/TBI).

Prophylactic immunosuppression was established with cyclosporine A (starting on day -1, taper from day +100 on) and methotrexate (on days +1, +3, +6 and +11) in patients with MAC. Patients with RIC received cyclosporine A (on day -4, taper from day +100 on) and mycophenolate (starting at day +1, taper from day +56 in patients with unrelated donors, and at day +28 in patients with related donors). GVHD prophylaxis with ATG (10 mg/kg/day on days -3 to -1) was given regularly to recipients of unrelated donor HCT (RIC and MAC), and to patients given RIC preparation before matched related donor HCT. ATG was not routinely given to recipients of matched related grafts receiving MAC preparation.

Classification and Grading of Clinical Parameters

Acute GVHD was classified as grade I to IV according to Glucksberg et al [34], and chronic GVHD was classified as low, moderate, or severe by the National Institutes of Health consensus criteria [35]. Patients were further grouped into those who were still receiving systemic immunosuppression versus those who were off immunosuppression at 1 year post-HCT.

Statistical Methods

Cox regression analysis was used to assess univariate and multivariate tests for loss of protective antibodies in subgroups of patients. Patients were grouped into (1) those age ≥50 years versus those age <50 years; (2) those with no or grade I acute GVHD versus those with grade II-IV acute GVHD; (3) those with no or mild chronic GVHD versus those with moderate/severe chronic GVHD; (4) those off pharmacologic immunosuppression at 1 year post-HCT, versus those receiving ≥1 drug at this time; and (5) those with lymphoproliferative neoplasms versus those with nonlymphoproliferative underlying disease.

We considered all 11 variables of interest for our multivariate analysis, according to Vittinghoff et al [36,37], for calculating outcome events per predictor variable. Differences between RIC and MAC were assessed using the chi-square test, with a P value <.05 considered statistically significant. Statistical analyses were conducted using SPSS version 21.0.0.0 (IBM, Armonk, NY) and Prism version 7 (GraphPad Software, La Jolla, CA).

RESULTS

Patient Characteristics

Three hundred thirty-one of 580 patients who underwent allogenic HCT between 2002 and 2015 at the University Hospital Zurich were included in this retrospective analysis. The median duration of follow-up was 5 years (range, .5 to 13 years). Owing to the lack of long-term follow-up, patients with early relapse post-HCT (within the first 12 months) and those who had received the MMR vaccine after HCT were excluded. In total, 249 patients were not considered for this study because of death due to disease relapse or treatment-related mortality (67% of the excluded patients died within 2 years post-HCT) or the need for salvage treatments (eg, chemotherapy, hypomethylating agents, donor lymphocyte infusions) because of relapsed disease (25%). The other patients who were excluded were either lost to long-term follow-up or received the MMR vaccine.

Table 1 displays patient characteristics, including age, sex, and underlying disease; details of the HCT procedure; and transplantation-related complications (acute and chronic GVHD) for the total cohort and separately for subgroups of patients who received MAC versus RIC conditioning. The median age was 48 years (range, 18 to 68 years). Patients

Table 1
Patient and Treatment Characteristics

Characteristic	Total Cohort (N = 331), n (%)		MAC (N = 173), n (%)		RIC (N = 158), n (%)		*P* Value*
Sex							
Female	155	(47)	81	(47)	74	(47)	.99
Male	176	(53)	92	(53)	84	(53)	
Age group							
≤30 yr	51	(15)	32	(18)	19	(12)	<.1
>30 <40 yr	59	(18)	45	(26)	14	(9)	
>40 <50 yr	80	(24)	56	(32)	24	(15)	
>50 <60 yr	100	(30)	38	(22)	62	(39)	
>60 yr	41	(12)	2	(1)	39	(25)	
Disease							
AML	154	(47)	96	(55)	58	(37)	NT
MDS	16	(5)	2	(1)	14	(9)	
CMML	2	(.6)	1	(.6)	1	(.6)	
CML	30	(9)	28	(16)	2	(1)	
PMF	17	(5)	0	(0)	17	(11)	
ALL	51	(15)	37	(21)	14	(9)	
NHL	26	(8)	7	(4)	19	(12)	
HD	8	(2)	0	(0)	8	(5)	
CLL	3	(1)	0	(0)	3	(2)	
MM	15	(5)	0	(0)	15	(9)	
CGD	6	(2)	1	(.6)	5	(3)	
SAA	3	(1)	1	(.6)	2	(1)	
Donor							
MRD	181	(55)	101	(58)	80	(51)	.15
MUD	150	(45)	72	(42)	78	(49)	
Graft type							
BM	68	(21)	48	(28)	20	(13)	<.01
MPB	263	(79)	125	(72)	138	(87)	
ATG							
No	113	(34)	99	(57)	14	(9)	<.01
Yes	218	(66)	74	(43)	144	(91)	
Acute GVHD							
Grade I	125	(38)	71	(41)	54	(34)	.06
Grade II	51	(15)	30	(17)	21	(13)	
Grade III	10	(3)	8	(5)	2	(1)	
Grade IV	10	(3)	5	(3)	5	(3)	
None	135	(41)	59	(34)	76	(48)	
Chronic GVHD							
Mild	116	(35)	59	(34)	57	(36)	.04
Moderate	36	(11)	27	(16)	9	(6)	
Severe	10	(3)	5	(3)	5	(3)	
None	169	(51)	82	(47)	87	(55)	

AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; CML, chronic myelogenous leukemia; PMF, primary myelofibrosis; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; CGD, chronic granulomatous disease; SAA, severe aplastic anemia; MRD, matched related donor; MUD, matched unrelated donor; NT, not tested.

* For comparison between MAC and RIC groups.

receiving MAC were younger than those receiving RIC (median, 41 years [95% confidence interval (CI), 39.9 to 44.9 years] versus 54 years [95% CI, 48.5 to 52.6 years]). Acute myelogenous leukemia was the most common indication for allogeneic HCT (47% of patients), followed by acute lymphoblastic leukemia (15%). Fifty-five percent of patients had an HLA-matched related donor, and 45% had an unrelated donor, including 41% with an HLA-matched unrelated donor and 5% with an HLA-mismatched donor. As expected, MPB was the graft source in the majority of patients (79%); 21% of patients were infused with BM. No recipients of cord blood were included in the analysis. The majority of patients (66%) received ATG as part of the GVHD prophylaxis regimen.

Of the 331 patients in the study cohort 173 (52%) received a MAC regimen and 158 (48%) received a RIC regimen. The most commonly used MAC regimen was Cy/TBI with or without ATG in 106 patients (61%), followed by Bu/Cy in 48 patients (28%). One hundred forty-six patients received RIC with Flu/Bu with or without ATG. Preparative regimens and GVHD prophylaxis varied according to the underlying disease, such patient characteristics as age and comorbidities, and type of donor (related or unrelated), and were also influenced by the time

period of the transplantation. In general, patients age ≥55 years with relevant comorbidities received a RIC regimen. Moreover, patients with certain diseases, such as lymphoproliferative diseases (including Hodgkin and non-Hodgkin lymphoma, myeloma, and chronic lymphocytic leukemia) and also some nonmalignant conditions (eg, chronic granulomatous disease, severe aplastic anemia) would typically receive RIC preparation at our center, regardless of age.

The rate of acute GVHD for the total cohort was 59% (38% grade I, 15% grade II, 6% grade III-IV). The rate of acute GVHD was lower in the patients who received RIC preparation compared with those who received MAC, but the difference was not statistically significant. The rate of chronic GVHD was 41% for the total cohort (35% mild, 11% moderate, and 3% severe). Following MAC preparation, there was a higher proportion of chronic GVHD that mostly manifested as moderate chronic GVHD.

Baseline Serostatus of Donors and Recipients Pretransplantation

The donor's serostatus at the time of stem cell donation was available only for recipients of related donor transplants, because this information is not routinely documented within the infectious disease markers provided with allografts from unrelated donors. Of the 171 donors with information on serostatus, 96% had antibodies against measles and 94% had protective titers against mumps and rubella. In most cases, both, donor and recipient were seropositive before transplantation (D+/R+). For measles, 91.8% of donor-recipient pairs were both seropositive (D+/R+), while only 4.1% of pairs were donor seropositive/recipient seronegative (D+/R-), 3.6% of pairs were donor seronegative/recipient seropositive (D-/R+), and .6% of pairs were both donor and recipient seronegative (D-/R-). For mumps, donor and recipient status was D+/R+ in 83.1%, D+/R- in 4.1%, D-/R+ in 10.5%, and D-/R- in 2.3%. For rubella, donor and recipient status was D+/R+ in 88.3%, D+/R- in 5.8%, D-/R+ in 5.8% and D-/R- in 0%.

Because for the majority of donor-recipient pairs, both donor and recipient were seropositive, and either the donor or recipient was seronegative in only a small number of pairs. Subgroups other than D+/R+ were too small to allow for statistical calculations with sufficient power to detect true differences between groups. Table 2 presents data on donor-recipient pairs that did not have both protective antibody titers against measles before transplantation. From these individual patients, it can be appreciated that the majority of patients who were seronegative before transplantation and received grafts from donors with immunity against measles remained seronegative at 1 year and beyond 2 years post-HCT. At 6 months post-HCT, some patients had positive antibody titers in the blood, some of which could be explained by intravenous immunoglobulin infusions. In contrast, when recipients were seropositive but donors seronegative before transplantation, recipients maintained some immune protection in the first months and years post-HCT, suggesting that without revaccination it is long-lived residual host plasma cells and/or memory B cells that continue to produce antibodies against measles and presumably other antigens post-HCT.

Assuming that also in recipients of unrelated grafts the majority of donors was seropositive for measles, mumps, and rubella, and it is host plasma cells that are the major producers of antibodies against these 3 viruses, we also included recipients of unrelated donor grafts in our analysis on the spontaneous decline of antibody titers post-transplantation.

Table 2
Influence of Donor/Recipient Serostatus on Immune Protection Against Measles Post-HCT

D/R Status Pre-HCT (N = 171)	6 mo	1 yr	≥2 yr
D-/R- (n = 1)			
#1 (+IVIG)	+	+	+
D+/R- (n = 7)			
#1	(-)	-	+*
#2 (+IVIG)	+	-	-
#3	-	-	-
#4	+	-	-
#5	+	+	+
#6	+	+	-
#7	(-)	-	NA
D-/R+ (n = 6)			
#1	+	+	+
#2	+	+	NA
#3	+	+	(-)
#4	+	+	+
#5	(-)	(-)	(-)
#6	+	+	+
D+/R+ (n = 157)			
. . .	132/141	114/128	77/100

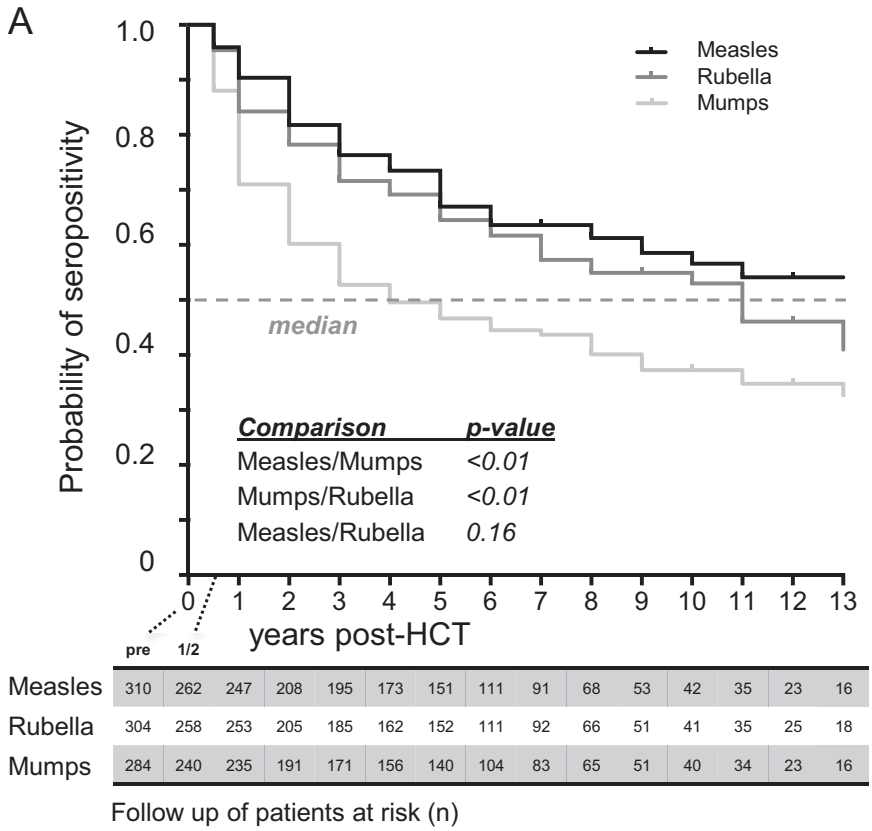
D indicates donor; R, recipient; (-), borderline titers; IVIG, intravenous immunoglobulin; NA, not available.
* Later negative.

Dynamics of Antibody Titers over Time

Most patients had protective antibody levels against measles, mumps, and rubella before transplantation (measles, 95%; mumps, 87%; rubella, 93.0%). Loss of protective antibody titers over time differed significantly among the 3 viruses. Titers against mumps dropped faster with loss of protection after a median of 4 years (95% CI, 2.4 to 5.6 years) compared with those against measles (median not reached), and rubella, in which loss of protection occurred after a median of 11 years (95% CI, 7.3 to 14.7 years; $P < .001$) (Figure 1A). Although no MMR-vaccination had been administered in this patient cohort, at 5 years post-HCT protective titers against measles and rubella were still present in 72% and 65% of all 331 patients, respectively, while 50% had lost protection against mumps (Figure 1B).

Dynamics of Antibody Titers in Relation to Age and Year of Birth

With the introduction of vaccines against measles, mumps and rubella in the 1960s the prevalence of these diseases decreased rapidly in Switzerland, with a steep decline in the early 1980s. Systematic reporting systems to provide exact numbers were only introduced in 1987, whereas earlier figures and numbers were estimates [9]. Since 1976 vaccination against measles in small children at the age of 12 months is part of the officially recommended vaccinations in Switzerland [9,38,39]. Thus, the majority of patients born after 1976 have been immunized against measles by vaccination rather than the wild-type viral pathogen. In contrast, the majority of individuals born before 1976 acquired anti-measles immunity by exposure to the wild-type virus because measles are highly contagious, and virtually all children were infected before age 10 years [40]. In univariate analysis 96% of patients born before 1976 were seropositive for measles before transplantation, and 92% of patients born after 1976 had protective antibody levels at this time.



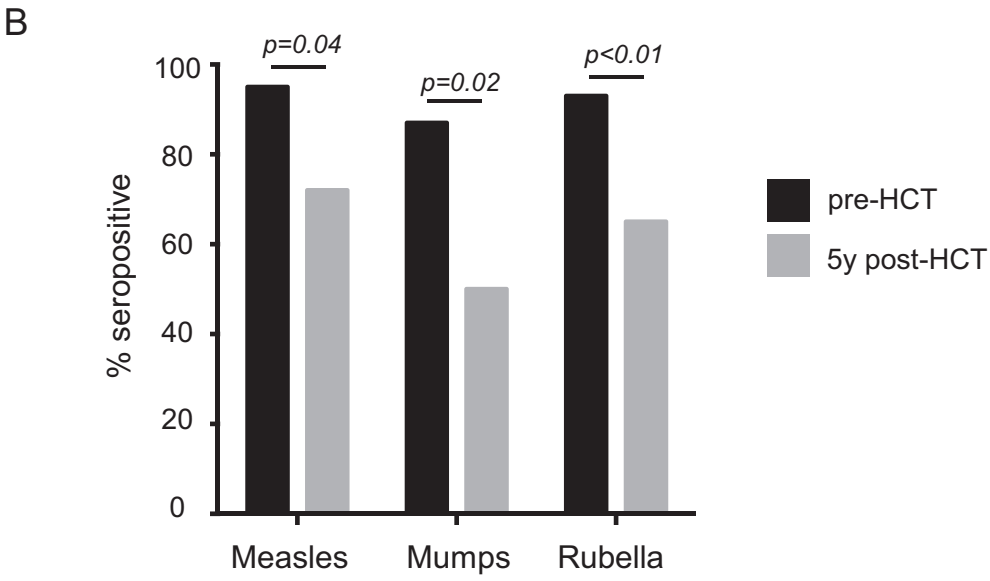


Figure 1. Dynamics of antibody titers against MMR. (A) Kaplan-Meier curves of patients with seropositivity for measles (n = 310), mumps (n = 284), and rubella (n= 304). (B) Proportions of patients seropositive for measles, mumps, and rubella before (black bars) and 5 years after (gray bars) HCT. *P* values were calculated by the chi-square test.

Considering only patients that were seropositive before HCT, we found that those born after 1976 had a more rapid loss of protective antibody titers (loss after a median of 4 years; 95% CI, 2.7 to 5.3 years) compared with those born before 1976 (median not reached; $P < .001$) (Figure 2A).

Vaccinating young children against mumps has been recommended since 1981 in Switzerland [39]. In the prevaccination era, the prevalence of mumps was high, and local endemic outbreaks were common [41]. Most individuals were infected as teenagers, but not everyone exposed to the virus

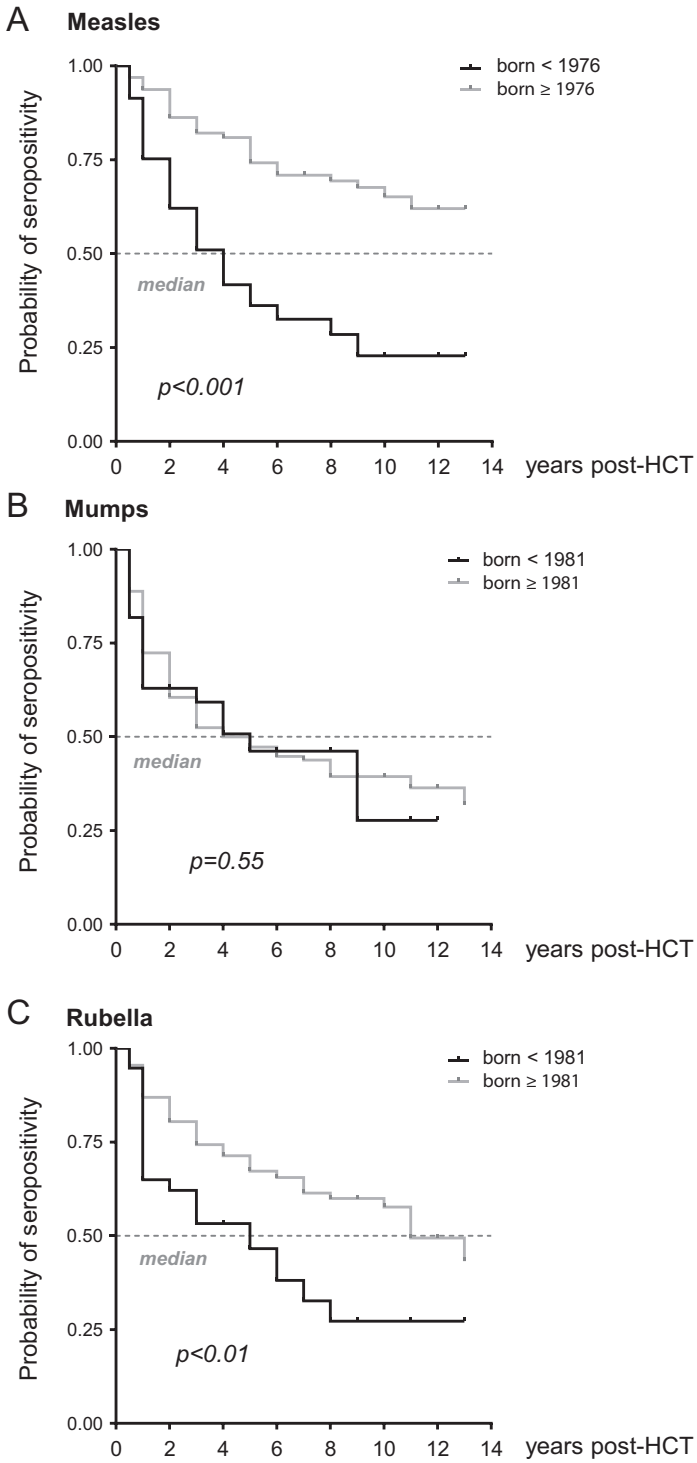


Figure 2. Dynamics of antibody titers in relation to age and year of birth. Shown are proportions of patients with protective antibodies against measles, displaying cohorts born before 1976 (n = 253) and after 1976 (n = 57) (A); mumps, displaying cohorts born before 1981 (n = 251) and after 1981 (n = 33) (B); and rubella, displaying cohorts born before 1981 (n = 265) and after 1981 (n = 39). *P* values were calculated by Cox regression analysis.

manifested signs of the disease (manifestation index ~80%) [41]. In our study cohort, 88% of patients born before 1981 had protective antibodies against mumps before HCT, and 79% of the younger patients born after 1981 were seropositive. Regarding the dynamics of the loss of antibody protection, the decline in immunity was faster for mumps than for measles and rubella but was similar in patients born before 1981 and those born after 1981 (median, 4 years [95% CI, 2.3 to 5.7 years] versus 4 years [95% CI, 1.2 to 6.9]; *P* = .55) (Figure 2B).

Vaccinations against rubella have been included in standard vaccination guidelines for all small children in 1981 in Switzerland [39]; however, the recommendation to vaccinate all girls at age 15 dates back to 1973 [38,39]. Therefore, many girls and young women born before 1981 have been

systematically vaccinated during adolescence (eg, in schools), and the current guidelines continue to recommend the vaccination boost against rubella in girls at age 15 years. Therefore, in our analysis, we were unable to distinguish those who were vaccinated against rubella from those who had been exposed to the wild-type virus. Moreover, rubella is less contagious than measles and has a lower manifestation index of approximately 60%, and naturally affects children around the age of 3 to 8 years [42,43]. The vast majority (96% of women and 91% of men) born before 1981 had protective antibodies against rubella before transplantation, and 94% of the younger women and 92% of the younger men had antibodies at this time point. Despite the aforementioned potential confounders, a less pronounced but in principle similar effect as seen in antibody titers against measles was observed for rubella. Patients born before 1981 displayed a slower loss of antibody protection compared with those born after 1981 (loss at a median of 11 years [95% CI, 8.6 to 13.4] versus 5 years [95% CI, 8.6 to 13.4]; $P < .01$) (Figure 2C), again suggesting that the natural confrontation with the wild-type virus results in a stronger and more persistent immune response than the attenuated vaccine.

Impact of Conditioning Intensity and Use of ATG on Antibody Titers

The gradual decrease of antibody titers over time was independent of donor serostatus. Together with the finding that even without MMR vaccination, protective antibody titers were detectable over months and years post-HCT, and considering the half-life of approximately 21 days for immunoglobulins, this suggests that antibodies are derived from residual host plasma and memory B cells. This is in line with the notion that plasma cells are highly resistant to radiotherapy and chemotherapy, even in the presence of full donor chimerism in the blood [2]. Therefore, we examined whether the intensity of the conditioning regimen had an influence on the course and decline of protective antibody titers over time. Compared with patients receiving RIC, those given MAC were younger ($P < .01$), more often received BM as an allograft ($P < .01$), and received less ATG ($P < .01$) (Table 1). Yet univariate analyses did not reveal any differences in antibody protection and its loss in patients receiving MAC or RIC regimens for allogeneic HCT conditioning in any of the 3 viruses examined (Figure 3). Similarly, ATG did not have an impact on the dynamics of antibody titers against measles, mumps, and rubella post-HCT in univariate analysis (Figure 4).

Antibody Titers in Patients with GVHD

Univariate analysis did not reveal any significant differences in the dynamics of antibody protection in patients with no or grade I acute GVHD compared with those with grade II- IV acute GVHD ($P = .09$ for measles, .50 for mumps, and .78 for rubella). In contrast, patients with no or mild chronic GVHD had more sustained antibody levels for measles post-HCT (median for loss of protection not reached) that were significantly higher than those in patients with moderate or severe chronic GVHD (loss of protection at a median of 6 years; 95% CI, 4.0 to 8.0 years; $P < .01$) (Figure 5A). There was no difference in loss of antibody protection against mumps and rubella between patients with no or mild chronic GVHD and those with moderate or severe chronic GVHD (Figure 5B and C).

Multivariate Analysis

To eliminate potential confounders, the impact of various factors was also assessed by multivariate analysis. Table 3 summarizes the respective hazard ratios and *P* values from 11 variables of interest. Patient-specific characteristics, such as sex and age, had no influence on antibody protection against any of the 3 viruses. Although age per se (>50 years and ≤50 years) was not associated with particular antibody dynamics, the separation of patients into cohorts born before and after implementation of the respective vaccinations into routine care of people living in Switzerland revealed statistically highly significant differences of immune protection against measles and rubella post-HCT also in multivariate analysis. The hazard ratio revealed a 2.9-fold increased risk for the loss of measles antibodies ($P > .001$) and a 2.4-fold increased risk for loss of rubella antibodies ($P < .001$) for the vaccinated cohorts, while there was no significant difference for mumps ($P = .24$).

The underlying disease (lymphoproliferative versus "other") had no influence on the loss of antibody protection over time, even though patients with lymphoproliferative diseases presumably were given heavily lymphocyte-depleting chemotherapies and immunotherapies before transplantation. Likewise, donor type (matched related versus unrelated), stem cell source (MPB versus BM), intensity of the conditioning regimen (MAC versus RIC), use of ATG, and the occurrence of acute GVHD had no association with the decline of antibody titers against measles, mumps, or rubella post-HCT. In contrast to univariate analysis, the absence or presence of chronic GVHD was not associated with persistence or loss of antibody protection in multivariate analysis. Similarly, there was no difference between patients who continued to receive pharmacologic immunosuppression at 1 year post-HCT compared with those who were off immunosuppressive treatment at that time (Table 3).

DISCUSSION

Infections contribute significantly to morbidity and mortality following allogeneic HCT [1,7]. Immunosuppressive pharmacologic treatments for prophylaxis and/or treatment of acute and chronic GVHD put patients at increased risk of infection. Moreover, GVHD itself is known to be associated with severe immune dysfunction [2]. In particular, B lymphopoiesis is impaired in some patients with GVHD, and hypogammaglobulinemia can persist for even years [2]. Revaccinations after allogeneic HCT provide some immune protection for this vulnerable patient population [44]; however, the efficacy of vaccinations administered early post-HCT, initiated at a time when many patients are still receiving immunosuppressive medications, is inconsistent and cannot be predicted [14]. Moreover, there are diseases for which no vaccinations are available or the vaccine is not recommended during the first 2 years post-HCT [8,9,17].

Here we studied the spontaneous course and decline of antibody titers against measles, mumps, and rubella in a cohort of allogeneic HCT recipients who were not revaccinated post-HCT to examine the influence of distinct clinical parameters, including underlying disease, intensity of the conditioning regimen, use of ATG, and other parameters, on immunity post-HCT. The preservation of protective antibodies strongly depended on the respective viral antigen, with the longest preservation of immunity for measles, followed by rubella. For measles and rubella, the immunity acquired by the natural disease lasted longer than that provided by vaccination with the attenuated virus. Immunity against mumps was poorly maintained for both wild-type infection and vaccine. For all 3 viruses, gradual loss of antibody protection occurred over time, but this decline was independent of conditioning intensity, use of ATG, underlying disease, donor type, and stem cell

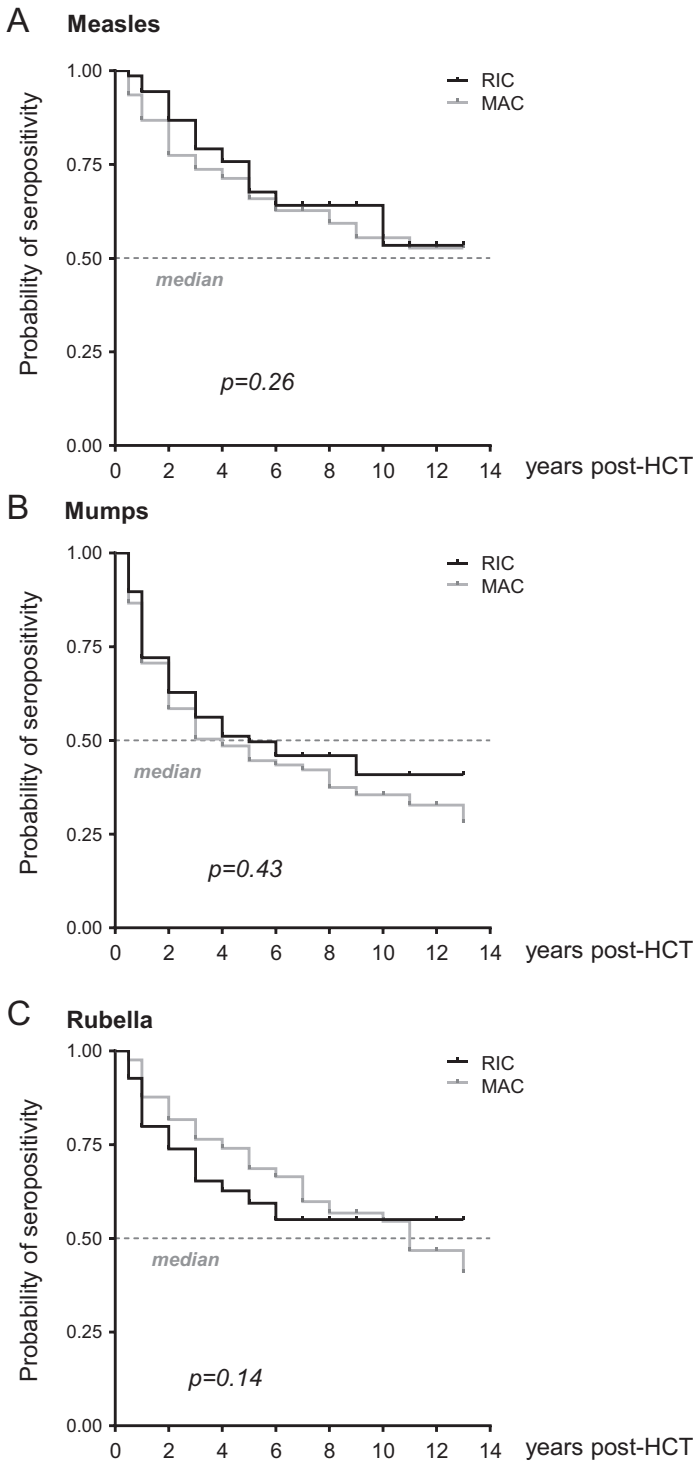


Figure 3. Impact of conditioning intensity on antibody titers. Shown are proportions of patients with protective antibodies against measles, displaying patients given MAC (n = 163) versus those given RIC (n = 147) (A); mumps, MAC (n = 156) versus RIC (n = 128) (B); and rubella, MAC (n = 167) versus RIC (n = 137) (C).

source. In our dataset, along with the source of immunization, only the presence of chronic GVHD had an impact on the loss of protective antibody titers.

The dynamics of antibody titers in transplant recipients following myeloablative conditioning before the era of regular ATG use have been described previously [28,45]. In 1994, Ljungman et al [29] reported the serostatus of antibody levels against measles, mumps, and rubella in a cohort of 124 patients following MAC preparation and allogeneic BM transplantation. Forty-eight patients were censored at the time of MMR vaccination. At 3, 5, and 7 years 47%, 27%, and 20%, respectively, of the remaining patients had sufficient immunity against measles, and proportions were similar for rubella but lower for mumps. Similar to our findings, the serostatus of the donor had no influence on post-transplantation antibody titers of the recipient. The sole significant parameter

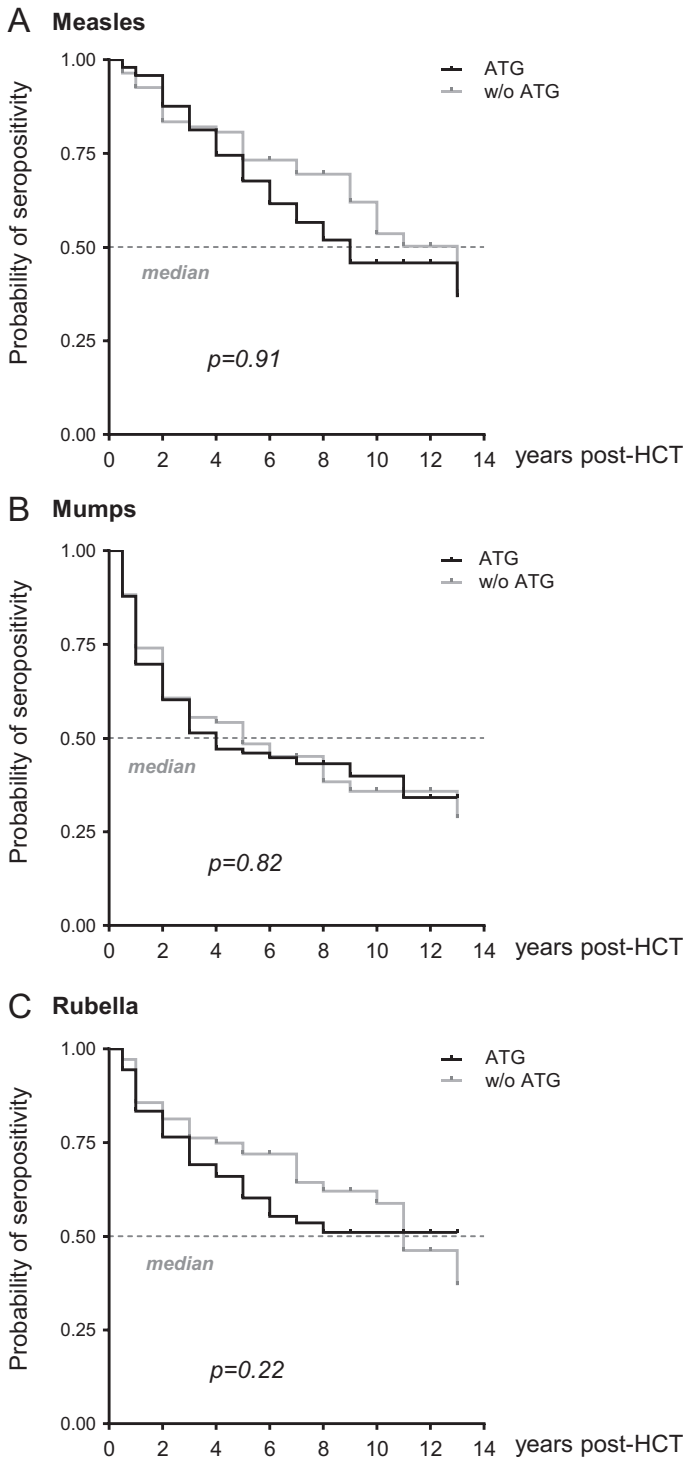


Figure 4. Impact of the use of ATG on antibody titers. Shown are proportions of patients receiving ATG versus those not receiving ATG within the conditioning regimen, displaying protective titers against measles (n = 202 versus n = 108) (A), mumps (n = 182 versus n = 102) (B), and rubella (n = 197 versus n = 107) (C).

determining post-transplantation immune protection was whether patients had been immunized by vaccination or by the natural disease. A decade later, Ljungman et al [45] updated their initial data and confirmed their previous findings on immunity against measles in a larger patient cohort, which still comprised mostly recipients of BM grafts who had received a MAC regimen. That study also identified acute GVHD grade II-IV and the use of MPB (rather than BM) as risk factors for loss of protective immunity against measles. Many children were included in this dataset (median age, 27.7 years; range, 1 to 63 years), the majority of whom were vaccinated against measles at 2 years post-HCT when there was no evidence of GVHD, whereas only few of the adults received the vaccine [45].

Our findings are in line with the important early work of Ljungman et al, as we also found that the group that was

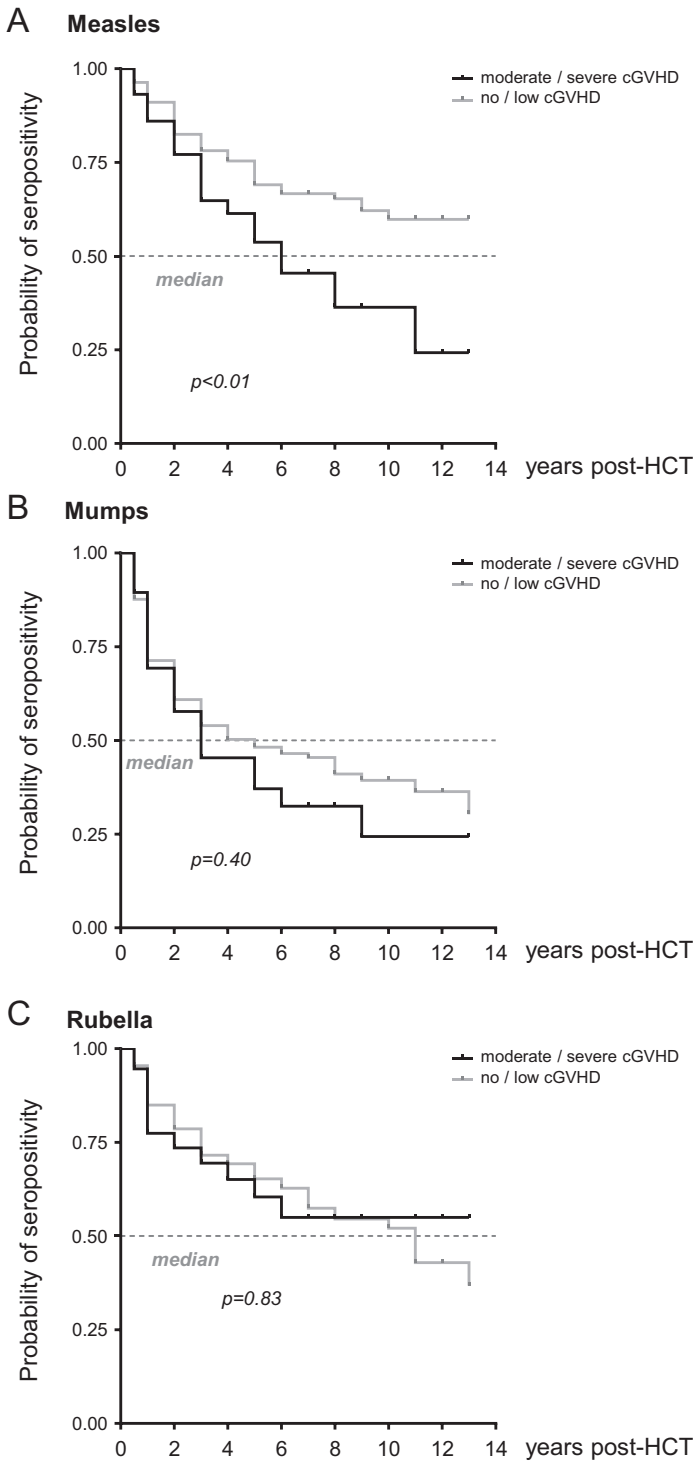


Figure 5. Antibody titers in patients with chronic GVHD. Shown are proportions of patients with protective antibodies against measles, displaying those with no or mild chronic GVHD (n = 270) versus those with moderate/severe chronic GVHD (n = 40) (A); mumps, with 246 patients with no/mild GVHD versus 38 with moderate/severe chronic GVHD (B); and rubella, with 267 patients with no/mild chronic GVHD versus 37 with moderate/severe chronic GVHD (C).

naturally immunized against measles and rubella had a slower loss of protective immunity post-transplantation. Further, our findings add data to the previous studies on a larger number of adult recipients of RIC versus MAC preparative regimens, MPB grafts, and ATG as part of the GVHD prophylaxis regimen. Our patient cohort was homogenous with regard to seropositivity before transplantation in the majority of patients and the exclusion of those patients who received the MMR vaccine post-HCT.

Contrary to our expectations, neither the intensity of the conditioning regimen nor the use of ATG had an influence on antibody titers post-transplantation. This was surprising, given that most specific antibodies appear to originate from residual host B or plasma cells, but may be due to the rather aggressive

Table 3
Multivariate Analysis of the Impact of Clinical Parameters on Post-HCT Immunity

Parameter	Measles		Mumps		Rubella	
	P Value	HR	*P* Value	HR	*P* Value	HR
Sex, female vs male	.14	.72 (.47-1.10)	.41	1.10 (.82-1.61)	.68	1.10 (.73-1.60)
Age group, ≤50 yr vs >50 yr	.36	.77 (.11-1.35)	.52	.87 (.57-1.33)	.47	1.20 (.72-2.00)
Immunization by disease vs vaccination	**<.001**	**2.90 (1.76-4.80)**	.24	.76 (.48-1.20)	**<.001**	**2.40 (1.40-4.00)**
Diseases, lymphoid vs other	.77	.93 (.60-1.47)	.70	1.08 (.73-1.59)	.85	.96 (.60-1.50)
Donor, MRD vs MUD	.24	1.41 (.80-2.51)	.80	1.06 (.67-1.60)	.62	.87 (.52-1.50)
Stem cell source, BM vs MPB	.55	.85 (.51-1.44)	.50	.86 (.55-1.34)	.50	.84 (.51-1.40)
Conditioning, MAC vs RIC	.76	1.08 (.66-1.78)	.19	1.42 (.84-2.40)	.37	1.20 (.76-2.10)
ATG, without vs with	.37	.75 (.40-1.40)	.59	1.12 (.74-1.70)	.79	1.10 (.59-2.00)
Acute GVHD, grade 0-I vs ...grade II-IV	.31	1.30 (.78-2.18)	.20	1.43 (.82-2.50)	.97	.99 (.60-1.63)
Chronic GVHD, no/mild vs moderate/severe	.24	1.50 (.76-2.94)	.17	.69 (.41-1.17)	.63	.83 (.41-1.71)
Immunosuppressive therapy, no vs yes	.89	.95 (.51-1.80)	.92	1.03 (.60-1.75)	.70	1.12 (.63-2.00)

Significant values are in bold type.
HR indicates hazard ratio.

nature of our RIC regimen. Unfortunately, we did not have a sufficient number of patients who received truly nonmyeloablative preparation and thus were not able to assess whether antibody protection would persist longer in these patients. Similarly, ATG did not have an influence on the persistence or loss of protective antibody titers post-HCT, even though ATG has been shown to have a substantial influence on reconstitution of the B cell compartment, with a significant decrease in memory B cells early during hematopoietic regeneration [46]. Our observations suggest that it is long-lived, residual host-type plasma cells that resist radiation and/or chemotherapy conditioning and continue to produce antibodies post-HCT. Accordingly, our study does not support the idea that specific donor immunity and immunologic memory can be transferred via B cells contained in the allograft.

Finally, as expected, we observed that chronic GVHD is associated with faster loss of immune protection. This may be due to immunosuppressive drugs, including cinluding calcineurin inhibitors and corticosteroids that are given for treatment of chronic GVHD. Moreover, the BM is a target organ of GVHD, and B cell lymphopenia is commonly observed in this group [2,47]. It is conceivable that the disruption of the BM microenvironment can also influence plasma cell function.

Studies like ours are of importance because society and populations, also from a health perspective, have changed over the past 2 decades. The proportion of the population that has been immunized by the wild-type virus is continually decreasing in Western society. In parallel, natural immune boosters for people with protective immunity occur less frequently with riddance of the viral diseases. The goal of all vaccination efforts was to achieve a vaccination rate >95%, thereby providing herd immunity to those who cannot be immunized, and ultimately completely eradicate and eliminate certain infectious diseases, including measles. Instead, we find ourselves in a situation in which the frequencies and sizes of outbreaks are increasing owing to the growing number of those who oppose vaccinations. This behavior puts vulnerable individuals, including transplant recipients, at enormous risk. Our findings suggest that younger patients who had acquired immunity against measles and immunity against rubella by vaccination pre-HCT may actually be at greater risk than older individuals when exposed to the virus. Moreover, accidental exposure may be particularly high in this age group, who are more likely than older individuals to be in contact with small children. At this point, the MMR vaccination is recommended at 2 years post-HCT when there is no evidence of GVHD. This recommendation is based on theoretical concerns regarding the use of attenuated live vaccines in immunocompromised individuals [8,17].

Very little data exist on the earlier administration of MMR vaccine in allogeneic HCT recipients. A recently published meta-analysis on outcomes following MMR vaccination given within the first 2 years post-HCT in a total of 152 HCT recipients reported no severe adverse events [48]. In this analysis, the efficacy of vaccinations varied widely across studies and among the respective vaccines, with an overall seroconversion rate of 86% to 100% for rubella, 33% to 46% for measles, and 29% to 80% for mumps [30,48-50]. The limited data in this study demonstrate a tendency toward lower efficiency for patients who were still under pharmacologic immunosuppression at the time of vaccination. Similarly, in another study that included adult patients receiving calcineurin inhibitors during vaccination, only 44% of the patients became seropositive after receipt of the measles vaccine [51]. Overall, the available data suggest that use of the MMR vaccine is safe before 2 years post-HCT, but the rate of seroconversion is unsatisfactory [48,49].

On the other hand, several reports on the severity of measles in HCT recipients and other immunocompromised patients have been published [24,27,30]. Therefore, the increasing risk of endemic occurrences of live-threatening infections versus the potential side effects and risks of a live-vaccine must be taken into consideration. The loss of immunity following allogeneic HCT will inevitably occur in a large proportion of patients. Serum antibodies should be regularly monitored, and depending on patient parameters (eg, age, vaccination status pre-HCT), the individual clinical situation (eg, GVHD, immunosuppression), and aspects of daily life (eg, contact with small children), the optimum time point for MMR vaccination should be determined, and routine administration in adults post-HCT implemented.

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edited the manuscript. A.M.S.M. wrote the manuscript and supervised the project.

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