Immunogenicity, Immunologic Memory, and Safety Following Measles Revaccination in HIV-Infected Children Receiving Highly Active Antiretroviral Therapy

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(See the editorial commentary by Maldonado, on pages 466–8.)

Background. Response rates and immunologic memory following measles vaccination are reduced in human immunodeficiency virus (HIV)–infected children in the absence of highly active antiretroviral therapy (HAART).

Methods. HIV-infected children 2 to <19 years old receiving HAART and with HIV loads <30,000 copies/mL, CD4% ≥15, and ≥1 prior measles-mumps-rubella vaccination (MMR) were given another MMR. Measles antibody concentrations before and 8, 32, and 80 weeks postvaccination were determined by plaque reduction neutralization (PRN). A subset was given another MMR 4–5 years later, and PRN antibody was measured before and 7 and 28 days later.

Results. At entry, 52% of 193 subjects were seroprotected (PRN ≥120 mIU/mL). Seroprotection increased to 89% 8 weeks postvaccination, and remained at 80% 80 weeks postvaccination. Of 65 subjects revaccinated 4–5 years later, 85% demonstrated memory based on seroprotection before or 7 days after vaccination. HIV load ≤400 copies/mL at initial study vaccination were determined by plaque reduction neutralization (PRN). A subset was given another MMR 4–5 years later, and PRN antibody was measured before and 7 and 28 days later.

Conclusions. Measles revaccination induced high rates of seroprotection and memory in children receiving HAART. Both endpoints were associated with HIV viral load suppression.

Clinical Trials Registration: NCT00013871 (www.clinicaltrials.gov).

Early in the human immunodeficiency virus (HIV) epidemic, it was recognized that measles can cause severe disease in HIV-infected children [1–3]. Prior to highly active antiretroviral therapy (HAART), HIV-infected children had reduced response rates, lower antibody titers, and more rapid antibody decline following measles vaccination; lack of recall responses; and vaccine failures [3–17]. Endemic measles and measles outbreaks pose a risk to HIV-infected children, and the HIV epidemic may complicate efforts for global measles control [18, 19]. Therefore, it is important to assess vaccine-induced immunity against measles in HIV-infected children in the context of HAART [20]. P1024 was a multicenter study of the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) designed to evaluate immunogenicity of vaccines in HIV-infected
children on HAART. P1061s was a substudy that evaluated immunologic memory following vaccination in P1024. This report focuses on immunogenicity, safety, and immunologic memory associated with measles vaccination.

METHODS

P1024 Population
HIV-infected children 2 to <19 years old at 39 US sites were eligible if they fit into the following strata based on pre-HAART nadir CD4% and screening CD4% (within 30 days before entry): stratum 1, <15% and <15%; stratum 2, <15% and ≥15%; stratum 3, 15 to <25% and ≥15%; stratum 4, ≥25% and ≥25%. Stratum 1 was excluded in the study of measles vaccination due to the requirement of a CD4% ≥15 for measles-mumps-rubella vaccine (MMR) administration. Other inclusion criteria included perinatal infection, treatment with the same HAART (≥3 antiretrovirals from ≥2 classes) regimen for ≥6 months, plasma HIV RNA viral load (VL) <30,000 copies/mL (Roche Amplicor Assay), and previous receipt of ≥1 dose of MMR, unless contraindicated by CD4 count [21–23].

P1024 Protocol
Informed consent was obtained and human experimentation guidelines of the US Department of Health and Human Services and participating institutions were followed. MMR (M-M-R II; Merck & Co, Whitehouse Station, NJ; 0.5 mL subcutaneously) was administered at the week 16 visit to subjects with a CD4% ≥15 and an absolute CD4 cell count ≥500/µL (age <6 years) or ≥200/µL (age ≥6 years) at the 2 preceding visits; pneumococcal polysaccharide vaccine was administered at the same visit and additional vaccines at other visits [21–23]. Measles antibody, plasma VL, and quantitative lymphocyte subsets were measured at entry and 8, 32, and 80 weeks postvaccination (study weeks 24, 48, and 96). Adverse reactions were assessed by diary and telephone calls or study visits 3, 7, 21, and 28 days postvaccination.

P1061s Protocol
Subjects enrolled in P1024 between June 2001 and March 2002 were eligible for P1061s, which enrolled between February 2006 and August 2006. Subjects who received MMR in P1024 with no grade ≥3 vaccine-related adverse event, did not receive MMR nor have proven measles infection since the conclusion of P1024, and had a CD4% ≥15 and an absolute CD4 cell count ≥200/µL on the 2 preceding measurements were eligible to have immunoglobin G memory response to measles vaccine evaluated in P1061s. MMR, hepatitis B, and pneumococcal (conjugate or polysaccharide) vaccines were administered at entry. Measles antibody was measured at entry and days 7 and 28, and VL and lymphocyte subsets were measured at entry. Adverse reactions were assessed by diary and telephone calls or study visits 3, 7, 21, and 28 days postvaccination.

Laboratory Assays and Immunologic Definitions
Measles neutralizing antibody concentrations were determined by plaque reduction neutralization (PRN) at the Centers for Disease Control and Prevention, Atlanta, GA, for P1024 serum samples and the Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD, for P1061s serum samples [24, 25]. PRN titers, defined as the serum dilution that reduced the number of plaques by 50%, were calculated using the Kärber method. A 1:100 dilution of World Health Organization (WHO) II reference serum (dilution concentration = 50 mIU/mL) was included in each run and all PRN titers were multiplied by a correction factor equal to 50 divided by the WHO II titer measured concurrently [25]. Seropositivity was defined as PRN concentration ≥8 mIU/mL and seroprotection as PRN concentration ≥120 mIU/mL [26, 27].

Vaccine response in P1024 was defined by PRN concentration ≥120 mIU/mL (protective antibody response) 8 weeks postvaccination. Antibody increases that were ≥4-fold and geometric mean concentration (GMC) were also evaluated. Subjects with antibody concentrations <8 mIU/mL were assigned a concentration of 2 mIU/mL. Immunologic memory in P1061s was defined on the basis of seroprotection (PRN concentration ≥120 mIU/mL) at P1061s entry or day 7 (protective memory response) or ≥4-fold rise in antibody concentration between P1061s entry and day 7 in subjects seropositive at entry. Primary response was defined in P1024 as ≥4-fold antibody rise 8 weeks postvaccination among subjects seronegative at entry and in P1061s as ≥4-fold antibody rise by day 28 postvaccination in subjects seronegative at P1061s entry.

Statistical Analysis
Subjects with results at entry and 8 weeks postvaccination (±4 weeks) were included in P1024 analyses. P1061s MMR recipients with results at entry, day 7 (day 5–15), and day 28 (±8 days) were included in P1061s analyses. Fisher’s exact test was used for comparison of proportions among groups, McNemar test for comparison of proportions between time points, t test for comparison of geometric mean concentrations (GMCs) among groups, and paired t test for comparison of GMCs between time points. Univariate regression analyses were performed to identify predictors of P1024 and P1061s measles antibody concentrations. Predictors with a P value <.1 were included in multivariate analyses. Stepwise regression was performed in the case of multiple collinear predictors.
Table 1. Characteristics of P1024 Measles Vaccine Recipients

| Characteristic                                      | Total (N = 193) | Stratum 2 (N = 63) | Stratum 3 (N = 73) | Stratum 4 (N = 57) | P Value
|-----------------------------------------------------|-----------------|--------------------|--------------------|--------------------|--------
| **Age at study MMR visit**                          |                 |                    |                    |                    |        
| Median (y)                                          | 9.8             | 10.9               | 10.0               | 6.8                | <.001  
| ≥7 y (%)                                            | 70              | 84                 | 77                 | 47                 | <.001  
| **Sex (%)**                                         |                 |                    |                    |                    |        
| F                                                    | 55              | 52                 | 56                 | 56                 | .90    
| **Race/ethnicity (%)**                              |                 |                    |                    |                    |        
| White non-Hispanic                                  | 13              | 14                 | 11                 | 14                 | .99    
| Black non-Hispanic                                  | 57              | 57                 | 56                 | 58                 |        
| Hispanic                                             | 30              | 29                 | 32                 | 28                 |        
| Asian/Pacific Islander                              | 1               | 0                  | 1                  | 0                  |        
| **CDC clinical classification (%)**                 |                 |                    |                    |                    |        
| N: nonsymptomatic                                    | 11              | 3                  | 12                 | 19                 | .001   
| A: mildly symptomatic                                | 34              | 35                 | 26                 | 44                 |        
| B: moderately symptomatic                            | 35              | 30                 | 45                 | 28                 |        
| C: severely symptomatic                              | 19              | 32                 | 16                 | 9                  |        
| **Pre-HAART nadir CD4%**                             |                 |                    |                    |                    |        
| Median                                              | 18              | 10                 | 19                 | 31                 | NA     
| <15 (%)                                              | 33              | 100                | 0                  | 0                  |        
| 15 to <25 (%)                                        | 38              | 0                  | 100                | 0                  |        
| ≥25 (%)                                              | 30              | 0                  | 0                  | 100                |        
| **Screening CD4%**                                   |                 |                    |                    |                    |        
| Median                                              | 34              | 30                 | 33                 | 40                 | NA     
| <15 (%)                                              | 1               | 0                  | 0                  | 0                  |        
| 15 to <25 (%)                                        | 11              | 22                 | 10                 | 4                  |        
| ≥25 (%)                                              | 87              | 76                 | 88                 | 96                 |        
| **CD4% at study MMR visit**                         |                 |                    |                    |                    |        
| Median                                              | 34              | 30                 | 35                 | 40                 | <.001  
| <15 (%)                                              | 1               | 2                  | 0                  | 0                  | .007   
| 15 to <25 (%)                                        | 13              | 22                 | 12                 | 4                  |        
| ≥25 (%)                                              | 87              | 76                 | 88                 | 96                 |        
| **Entry CD19%**                                      |                 |                    |                    |                    |        
| Median                                              | 19              | 18                 | 19                 | 19                 | .60    
| **CD19% at study MMR visit**                        |                 |                    |                    |                    |        
| Median                                              | 18              | 18                 | 18                 | 19                 | .42    
| **HIV RNA level at study MMR visit**                |                 |                    |                    |                    |        
| Median, copies/mL                                    | 278             | 386                | 260                | 245                | .008   
| ≤400 copies/mL (%)                                   | 63              | 51                 | 62                 | 77                 | <.001  
| 401–5000 copies/mL (%)                              | 22              | 19                 | 32                 | 14                 |        
| >5000 copies/mL (%)                                  | 15              | 30                 | 7                  | 9                  |        
| **Interval from last previous MMR to study MMR visit**|                 |                    |                    |                    |        
| Median (y)                                          | 4.8             | 6.4                | 5.0                | 3.4                | <.001  
| >2 y (%)                                             | 84              | 89                 | 86                 | 77                 | .20    
| **Number of MMR vaccines prior to entry (%)**       |                 |                    |                    |                    |        
| 1                                                    | 20              | 16                 | 16                 | 30                 | .27    
| 2                                                    | 70              | 71                 | 75                 | 61                 |        
| 3                                                    | 10              | 13                 | 13                 | 9                  |        

Data are percentages of subjects, unless otherwise indicated. Immunologic strata are as follows: stratum 2, pre-HAART nadir CD4 cell percentage <15% and screening CD4 cell percentage ≥15%; stratum 3, pre-HAART nadir CD4 cell percentage 15% to <25% and screening CD4 cell percentage ≥15%; stratum 4, pre-HAART nadir CD4 cell percentage ≥25% and screening CD4 cell percentage ≥25%. No subjects in study stratum 1 (pre-HAART nadir CD4 cell percentage <15% and screening CD4 cell percentage <15%) were included in the study of measles vaccination due to the requirement of a CD4% ≥15% for MMR administration.

Abbreviations: CDC, Centers for Disease Control and Prevention; HAART, highly active antiretroviral therapy; MMR, measles-mumps-rubella vaccine; NA, not applicable.

* Fisher’s exact test for categorical variables and Kruskal–Wallis test for continuous variables.
RESULTS

P1024 Study Population

Of 263 subjects enrolled, 226 received MMR per protocol (Table 1; flow diagram online). Remaining subjects had inclusion/ exclusion criteria violations (8), did not qualify for MMR based on CD4 criteria (19), did not receive MMR per protocol (9), or had other violations (1). One hundred ninety-three who received MMR per protocol had measles serology pre- and 8 weeks post-MMR vaccination and comprised the P1024 analysis group.

Table 2. Measles Serologic Status Before and After Measles Vaccination in P1024

<table>
<thead>
<tr>
<th>Endpoint and Study Time Point</th>
<th>All Strata Combined</th>
<th>Stratum 2</th>
<th>Stratum 3</th>
<th>Stratum 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent with PRN ≥8 mIU/mL at entry</td>
<td>83 (193)</td>
<td>76 (63)</td>
<td>81 (73)</td>
<td>95 (57)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(77–88)</td>
<td>(64–86)</td>
<td>(70–89)</td>
<td>(85–99)</td>
</tr>
<tr>
<td>Percent with PRN ≥120 mIU/mL</td>
<td>52 (193)</td>
<td>37 (63)</td>
<td>49 (73)</td>
<td>74 (57)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(45–60)</td>
<td>(25–50)</td>
<td>(37–61)</td>
<td>(60–84)</td>
</tr>
<tr>
<td>8 wk post-MMR (study week 24)</td>
<td>89 (193)</td>
<td>86 (63)</td>
<td>89 (73)</td>
<td>93 (57)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(84–93)</td>
<td>(75–93)</td>
<td>(80–95)</td>
<td>(83–98)</td>
</tr>
<tr>
<td>32 wk post-MMR (study week 48)</td>
<td>82 (185)</td>
<td>82 (61)</td>
<td>81 (69)</td>
<td>82 (55)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(75–87)</td>
<td>(70–91)</td>
<td>(70–90)</td>
<td>(69–91)</td>
</tr>
<tr>
<td>80 wk post-MMR (study week 96)</td>
<td>80 (179)</td>
<td>76 (59)</td>
<td>77 (66)</td>
<td>89 (54)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(74–86)</td>
<td>(63–86)</td>
<td>(65–87)</td>
<td>(77–96)</td>
</tr>
</tbody>
</table>

Study MMR vaccine administered at study week 16. Immunologic strata are as follows: stratum 2, pre-HAART nadir CD4 cell percentage <15% and screening CD4 cell percentage ≥15%; stratum 3, pre-HAART nadir CD4 cell percentage 15% to <25% and screening CD4 cell percentage ≥15%; stratum 4, pre-HAART nadir CD4 cell percentage ≥25% and screening CD4 cell percentage ≥25%.

Abbreviations: CI, confidence interval; HAART, highly active antiretroviral therapy; MMR, measles-mumps-rubella vaccine; PRN, plaque reduction neutralization antibody concentration.

a) Week 0 serological results were included if obtained any time prior to study MMR vaccine administration. Serological results at 8, 32, and 80 weeks post-vaccination (study weeks 24, 48, and 96) were included if they were obtained within windows of ±4, ±8, and ±12 weeks, respectively.

b) P <.01 (Fisher’s exact test) for the difference of the proportions with measles antibody concentration ≥8 mIU/mL among immune strata.

c) P <.001 (Fisher’s exact test) for the difference of the proportions with measles antibody concentration ≥120 mIU/mL among immune strata. Pairwise comparisons between stratum 4 and strata 2 and 3 were statistically significant (P <.007), but pairwise comparisons between strata 2 and 3 were not.

d) P <.004 (McNemar test) for the comparison of measles antibody concentration ≥120 mIU/mL vs the previous time point.

e) P <.04 (McNemar test) for the comparison of the proportions of subjects with measles antibody concentration ≥120 mIU/mL at 32 weeks postvaccination (study week 48) and 80 weeks postvaccination (study week 96) vs week 0.

f) P <.01 (t test) for the difference among immune strata. Pairwise comparisons between stratum 4 and strata 2 and 3 were statistically significant (P <.001), but pairwise comparisons between strata 2 and 3 were not.

g) P <.02 (paired t test) for the comparison of geometric mean antibody concentration vs the previous time point.

h) P <.01 (paired t test) for the comparison of geometric mean antibody concentration at 32 weeks postvaccination (study week 48) and 80 weeks postvaccination (study week 96) vs week 0.
Eight, 32, and 80 weeks postvaccination correspond to study weeks 24, 38, and 96, respectively. Abbreviations: Ndr, nadir; Scr, screening.

The GMC increased from entry to 8 weeks postvaccination. By 8 weeks postvaccination, GMCs of strata 2 and 3, but not of stratum 4, remained significantly greater than baseline; all exceeded the seroprotective level. GMCs of stratum 4 were consistently higher than those of strata 2 and 3, but differences were not significant at 8 and 32 weeks postvaccination and marginally significant at 80 weeks postvaccination.

**P1024 Measles Antibody Concentrations and VL**

At entry, the proportion of subjects with PRN ≥120 mIU/mL was inversely related to VL group, but differences were not significant (Figure 2). Eight weeks postvaccination, this inverse relationship was significant and differences remained significant at 32 and 80 weeks postvaccination; pairwise comparisons revealed differences between VL ≤400 copies/mL versus VL 401–5000 and >5000 copies/mL, but not between the latter 2 groups. GMCs varied inversely with VL at entry and each time point following vaccination. Pairwise analyses showed that subjects with VL ≤400 copies/mL had a higher GMC versus those with >5000 copies/mL at entry and versus those with 401–5000 or >5000 copies/mL postvaccination; differences between the latter groups were not significant.

**Predictors of P1024 Entry Measles Antibody Concentration**

Univariate analyses identified the following predictors of higher entry antibody concentration: age <7 years; nadir CD4% prior to HAART ≥25; CD4% ≥25 and VL ≤400 copies/mL at the last MMR vaccination prior to entry; shorter interval from last MMR to entry; shorter duration of the entry HAART regimen; immune stratum 4; and CD4% ≥25, VL ≤400 copies/mL, or 401–5000 copies/mL, and higher total lymphocyte count at entry. Sex, race/ethnicity, and CD19% at entry were not associated with entry antibody concentration. In a multivariate analysis, age <7 years and entry VL ≤400 copies/mL or 401–5000 copies/mL remained associated with higher entry antibody concentration (P ≤.02).

**Predictors of P1024 Measles Antibody Concentration 8 Weeks Postvaccination**

After adjusting for baseline antibody concentration, VL ≤5000 copies/mL at entry (vs >5000 copies/mL) and VL ≤400 copies/mL at the MMR study visit (vs 401–5000 copies/mL and >5000 copies/mL) were associated in univariate analyses with higher antibody concentration 8 weeks after MMR vaccination. Longer duration of the entry HAART regimen was marginally associated with higher antibody concentration (P = .06). Age; sex; race/ethnicity; nadir CD4% prior to HAART; interval from last previous MMR to study MMR visit; immune stratum; CD4% at entry; and CD4%, CD19%, and total lymphocyte count at the MMR study visit were not associated with the antibody concentration 8 weeks after vaccination. Multivariate analysis found only VL ≤400 copies/mL at the MMR study visit (vs 401–5000 copies/mL and >5000 copies/mL) associated with higher measles antibody.
concentration 8 weeks postvaccination, after adjusting for baseline antibody concentration \((P \leq .03)\).

**Figure 2.** P1024 proportion of subjects with antibody concentration \(\geq 120\) mIU/mL according to P1024 HIV RNA group and P1024 geometric mean concentration (GMC) according to P1024 HIV RNA group. Proportion of subjects with protective measles neutralizing antibody concentrations (top panel, A) and GMCs of measles neutralizing antibody (bottom panel, B) are shown at each P1024 study visit according to P1024 HIV viral load group. The proportion with protective \((\geq 120\) mIU/mL) antibody concentrations was higher for subjects with \(\leq 400\) copies/mL than for each of the other viral load groups at each time point after study MMR vaccination, administered at study week 16. The GMC of subjects with a viral load \(\leq 400\) copies/mL was higher than that of subjects with a viral load \(>5000\) copies/mL at entry \((P = .004)\) and higher than that of each of the other 2 viral load groups at each time point after study MMR vaccination \((P \leq .004)\). Time point 0 on the x-axis reflects results of serum samples obtained at entry. Eight, 32, and 80 weeks postvaccination correspond to study weeks 24, 48, and 96, respectively. Bars represent 95% confidence intervals.

**P1061s Study Population**

Of 224 eligible P1024 subjects, 101 were enrolled in P1061s (flow diagram online). Of these, 80 met inclusion criteria, fulfilled CD4 criteria to receive MMR in P1024 and P1061s, lacked grade \(\geq 3\) adverse events following the P1024 dose of MMR, and received MMR per protocol in both studies; 65 of 80 had entry, day 7, and day 28 measles antibody data and were included in P1061s analyses. Their characteristics at P1024 entry and rates of measles seroprotection and GMCs at each P1024 time point were similar to those of the entire P1024 analysis group. Their median CD4% at P1061s entry was 35%; 12% had a CD4% \(15\%–<25\%\) and 88% had a CD4% \(\geq 25\%\), and for all 65, the CD4% at P1061s entry was consistent with their original P1024 immunologic stratum assignment (32%, 38%, and 29% in strata 2–4, respectively).

Ninety-five percent were on HAART, 3% were on non-HAART antiretroviral therapy, and 2% were not receiving antiretroviral treatment. Sixty-eight percent had a VL \(\leq 400\) copies/mL, 18% were between \(401–5000\) copies/mL, and 13% had \(>5000\) copies/mL. Median time from P1024 MMR vaccination to P1061s entry was 4.24 years (interquartile range, 4.13–4.38 years).

**P1061s Measles Antibody Concentrations**

Ninety-eight percent were seropositive and 75% had seroprotective antibody concentrations at entry, higher than the seroprotection rate at P1024 entry and only slightly lower than seroprotection rates following P1024 vaccination (Table 3). At day 7, 83% were seroprotected, and 85% had protective memory defined by PRN concentrations \(\geq 120\) mIU/mL at entry or day 7. By day 28, 95% achieved seroprotective antibody concentrations. Of the 64 subjects seropositive at entry, only 5% demonstrated memory defined by \(\geq 4\)-fold antibody rise between entry and day 7, while 25% manifested \(\geq 4\)-fold antibody rises between entry and day 28. The single subject seronegative at P1061s entry (and before and after P1024 revaccination) experienced a \(\geq 4\)-fold seroprotective response by day 28.

Differences according to immune strata in the percentages seroprotected at entry, day 7, or day 28 or in the proportions with memory (76%, 88%, 89% for seroprotection at entry or day 7 and 5%, 4%, 5% for \(\geq 4\)-fold rise between entry and day 7, for strata 2–4, respectively) were not significant. The trend toward higher GMCs at entry and day 7 with increasing immune stratum was also not significant (Figure 3). There were no differences based on P1061s VL in the percentages seroprotected at entry, day 7, or day 28 or in the proportions with memory, or in GMCs (data not shown). However, there were differences according to VL at the P1024 MMR vaccination visit in rates of seroprotection at day 7 (91% vs 67% for VL \(\leq 400\) copies/mL vs \(>400\) copies/mL, respectively, \(P = .03\); Fisher’s exact test) and memory defined as seroprotection at
day 0 or day 7 (91% vs 71%, $P = .06$) and in GMCs at P1061s entry and day 7 (Figure 4).

Univariate analysis identified the following predictors of higher day 7 measles antibody concentration in P1061s: antibody concentration 8 weeks post-P1024 MMR vaccination ($P < .001$), antibody concentration at P1061s entry ($P < .001$), and VL ≤ 400 copies/mL at P1024 MMR vaccination ($P = .007$). Age; race; sex; interval from previous MMR to P1024 vaccination; nadir CD4% prior to HAART; duration of the entry HAART regimen prior to P1024 vaccination; immune stratum; measles antibody concentration at P1024 entry; total lymphocyte count, CD4%, and CD19% at the P1024 MMR visit; being on HAART at P1061s entry; and total lymphocyte count, CD4%, CD19% and VL ≤ 400 copies/mL at P1061s entry were not significantly associated with day 7 antibody concentration.

Safety (P1024 and P1061s)

Among 193 subjects in the P1024 dataset, 4 (2%) experienced grade 3/severe systemic events (3 fever, 1 fatigue) judged possibly or probably related to MMR. One MMR recipient with insufficient serologic data experienced grade 3 fever and pharyngitis possibly or probably related to vaccination. No grade

### Table 3. Measles Serologic Status Before and After Measles Vaccination in P1061s

<table>
<thead>
<tr>
<th>Endpoint and Study Time Point</th>
<th>All Strata Combined</th>
<th>Stratum 2</th>
<th>Stratum 3</th>
<th>Stratum 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>65</td>
<td>21</td>
<td>25</td>
<td>19</td>
</tr>
<tr>
<td>Percent with PRN ≥ 8 mIU/mL on day 0 (95% CI)</td>
<td>98 (92–100)</td>
<td>95 (76–100)</td>
<td>100 (86–100)</td>
<td>100 (82–100)</td>
</tr>
<tr>
<td>Percent with PRN ≥ 120 mIU/mL</td>
<td>Day 0 (MMR administration) (95% CI)</td>
<td>75 (63–85)</td>
<td>71 (48–89)</td>
<td>72 (51–88)</td>
</tr>
<tr>
<td></td>
<td>Day 7 postvaccination (95% CI)</td>
<td>83 (72–91)</td>
<td>76 (53–92)</td>
<td>88 (69–97)</td>
</tr>
<tr>
<td></td>
<td>Day 28 postvaccination (95% CI)</td>
<td>95 (87–99)</td>
<td>95 (76–100)</td>
<td>92 (74–99)</td>
</tr>
<tr>
<td>Percent with ≥4-fold rise in measles antibody concentration among subjects seropositive at entry</td>
<td>Day 0 to day 7 (95% CI)</td>
<td>5 (1–13)</td>
<td>5 (0–25)</td>
<td>4 (0–20)</td>
</tr>
<tr>
<td></td>
<td>Day 0 to day 28 (95% CI)</td>
<td>25 (15–37)</td>
<td>20 (6–44)</td>
<td>32 (15–54)</td>
</tr>
<tr>
<td>Geometric mean antibody concentration, mIU/mL (95% CI)</td>
<td>Day 0 (MMR vaccination)</td>
<td>295 (213–409)</td>
<td>238 (122–465)</td>
<td>316 (182–547)</td>
</tr>
<tr>
<td></td>
<td>Day 28 postvaccination</td>
<td>834 (636–1093)</td>
<td>839 (503–1401)</td>
<td>812 (486–1358)</td>
</tr>
</tbody>
</table>

Immunologic strata are as follows: stratum 2, pre-HAART nadir CD4 cell percentage <15% and screening CD4 cell percentage ≥15%; stratum 3, pre-HAART nadir CD4 cell percentage 15% to <25% and screening CD4 cell percentage ≥15%; stratum 4, pre-HAART nadir CD4 cell percentage ≥25% and screening CD4 cell percentage ≥25%. No statistically significant differences according to immune stratum were observed.

Abbreviations: CI, confidence interval; MMR, measles-mumps-rubella vaccine; PRN, plaque reduction neutralization antibody concentration.

a Day 7 serological results were included if they were obtained within days 5–15 after day 0 and day 28 serological results were included if they were obtained within a window of ±8 days.

b $P = .02$ (McNemar test) for the comparison of the proportion of subjects with measles antibody concentration ≥120 mIU/mL at day 28 vs day 7 and $P ≤ .001$ (McNemar test) for the comparison of the proportions of subjects with measles antibody concentration ≥120 mIU/mL at day 28 vs day 0.

c $N = 64$ subjects seropositive at entry, including 20 in stratum 2, 25 in stratum 3, and 19 in stratum 4.

d $P ≤ .03$ (paired t test) for the comparison of geometric mean antibody concentration vs the previous time point.

e $P ≤ .004$ (paired t test) for the comparison of geometric mean antibody concentration at day 28 vs day 0.
In contrast, measles seroprotection increased to 89% after revaccination in the context of HAART. Furthermore, we observed ≥4-fold antibody rises in 44% of subjects, including primary ≥4-fold responses in 78% of subjects seronegative at entry. Seroprotection rates of 93% in the highest immune stratum and 95% among subjects with VL <400 copies/mL rival ≥95% protection rates following 2 vaccine doses in HIV-uninfected children [33]. Although antibody concentrations fell beyond 8 weeks postvaccination, seroprotection fell only slightly, with 80% seroprotected 8 weeks postvaccination. These findings are consistent with smaller studies that showed response rates of 60%–90% in children revaccinated against measles after HAART [6, 28, 29, 31–32]. Some studies demonstrated persistence of antibody for 12–36 months, while others reported rapid declines and loss of detectable levels [31, 34].

In P1061s, 75% had measles antibody concentrations ≥120 mIU/mL at entry, only slightly lower than the percentage seroprotected at the conclusion of P1024. Memory was further evidenced by 85% having seroprotective antibody levels at entry or 7 days after the P1061s MMR dose. Parenthetically, the P1061s MMR dose likely stimulated a primary response in the single seronegative subject and produced antibody increases in several others who had antibody concentrations below the protective level at entry and day 7, indicating that an additional dose may induce and/or boost immunity in subjects who, despite HAART, had no or limited response or lacked memory following previous vaccination. Overall, we observed a surprisingly high seroprotection rate of 95% by day 28. Although studies are mixed as to persistence of memory following revaccination while on HAART, our finding of persistent memory 4–5 years after measles revaccination is consistent with studies which demonstrated protective antibody levels and measles-specific memory B cells several years after vaccination while on HAART [32, 34–36].

Responses to MMR in P1024 and detection of immunologic memory in P1061s were greatest in children with an undetectable VL at the time of P1024 vaccination and were not related to CD4% or CD19% measurements. For inactivated vaccines studied in P1024, both concurrent CD4% and VL were significant predictors of response [21–23]. Other studies found that response to measles and varicella-zoster vaccines in children on HAART was related more to suppression of VL than to CD4 values [29, 37, 38]. This suggests that responses to live vaccines may be particularly influenced by adverse effects of HIV replication on number and function of B (including memory cells) and T cells. HAART may mitigate these effects, reinforcing the importance of vaccinating when VL is maximally suppressed [14, 32, 35, 36, 39].

We observed varying immune responsiveness to different vaccines in the same population of HIV-infected children. P1024 responses were high for measles and pneumococcal conjugate vaccines, modest for pertussis vaccine, and weak for

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**DISCUSSION**

At P1024 entry, approximately half of subjects lacked protective antibody levels against measles, despite all having received MMR previously and 80% having received ≥2 doses. This reflects that most subjects were >2 (median 4.8) years removed from their last MMR, and many likely were not receiving HAART when previously vaccinated. The low seroprotection rate is consistent with low response rates to measles (re)vaccination (25%–75%) and of measles seroprevalence (5%–75%) in HIV-infected children not on HAART and reflects poor primary responses, impaired avidity, rapid waning of immunity, and defective memory [4–17]. Low levels and ongoing decline of measles antibody continue to be characteristic of children treated with HAART subsequent to vaccination [9, 28–32]. The low seroprotection rate prior to revaccination is also consistent with low antibody concentrations for *Streptococcus pneumoniae, Bordetella pertussis*, and hepatitis B virus at P1024 entry [21–23], and the assessment that HAART is unlikely to restore immunologic memory for vaccinations administered prior to HAART [32].

≥3 adverse events were reported among 10 other subjects who received MMR vaccine but were excluded from analyses for protocol violations (4) or not fulfilling protocol CD4 criteria (6). No grade ≥3 adverse events related to MMR were reported in P1061s. No vaccine-related potentially life-threatening events or deaths were observed.

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**Figure 4.** P1061s geometric mean concentration (GMC) according to P1024 HIV RNA group. GMCs of measles neutralizing antibody are shown at each P1061s study visit for all subjects combined and according to HIV viral load <400 copies/mL vs >400 copies/mL at the P1024 MMR visit. The GMC of subjects with a viral load <400 copies/mL was higher than that of subjects with a viral load >400 copies/mL at P1061s entry and day 7. MMR vaccine was administered on P1061s day 0.
hepatitis B virus vaccine [21–23]. In P1061s, immunologic memory was demonstrated 4–5 years later for measles and pneumococcal vaccines, but in only a minority after hepatitis B vaccination [23,40]. This demonstrates variability in response and memory induction to different immunogens in HIV-infected children on HAART.

Limitations of our study included that we did not know what antiretroviral therapy subjects may have received when given MMR prior to P1024, whether subjects who lacked measles antibody at entry had responded to previous MMR, and if subjects who lacked seroprotective antibody levels had ever attained seroprotective levels. Antibody measurements 8 weeks after P1024 vaccination may have missed peak responses. In P1061s, despite a high rate of memory based on seroprotection, far fewer subjects fulfilled the memory criterion based on ≥4-fold antibody rise by day 7 postvaccination, and only a minority manifested ≥4-fold rises by day 28. This suggests that neutralizing antibody present at entry in the majority was sufficient to inhibit replication of vaccine virus, consistent with memory but precluding rapid, large anamnestic responses, similar to HIV-uninfected children who most often do not attain ≥4-fold rises after revaccination. It is also consistent with our finding in P1024 that ≥4-fold rises were more frequent among initially seronegative subjects and that the magnitude of antibody rises tended to be lower in subjects with higher entry antibody concentrations (data not shown). Thus, the ≥4-fold rise criterion likely underestimated memory, and memory B cell assays would have been informative. It is also possible that, despite HAART, deficiencies in B cell memory and/or CD4 cell help in HIV-infected children limit the rate and/or magnitude of secondary antibody responses [32]. If memory kinetics are delayed, definitions of memory focused on seroprotection and ≥4-fold antibody rise by day 7 may have underestimated true proportions with memory, and antibody increases between days 7 and 28 in subjects seropositive at entry may have represented memory responses. Furthermore, it can be argued that all subjects seropositive at entry were immunologically primed and had some memory. Finally, the number of subjects in the datasets may have limited the power to discern predictors of vaccine response other than VL, multiple comparisons may have introduced chance associations in our exploratory analyses of predictors, and follow-up duration may have been inadequate to detect late adverse events (eg, pneumonitis or encephalitis).

Geographic overlap of the HIV epidemic and endemic measles transmission place HIV-exposed and HIV-infected children at risk [39,41]. Infants born to HIV-infected mothers, regardless of whether they are HIV-infected, are at increased risk of measles infection, possibly related to lower levels of maternal measles antibody. HIV-infected children are at high risk of measles infection due to poor immunologic memory despite prior vaccination [39]. Measles morbidity and mortality are enhanced in HIV-infected populations, even among children receiving HAART [18,19]. Although modeling suggests that the HIV epidemic has had limited impact on dynamics of measles transmission in developing countries because of the high mortality associated with HIV in the absence of antiretroviral therapy, the HIV epidemic may be expected to contribute more heavily to an increase in measles with escalating use of antiretroviral therapy and survival of HIV-infected children, if unprotected against measles [20]. Therefore, protecting HIV-infected children against measles with vaccination while on HAART is important not only for their own health, but also for global measles control. The present study reinforces the safety and potential value of measles (re)vaccination, when administered with an adequate CD4% in the context of HAART, to achieve high response rates with persisting immunologic memory [18,20,39,41]. This strategy is of great importance to areas threatened simultaneously by both highly pathogenic viruses.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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