Supplementary appendix

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Supplement to: Sankaranarayanan R, Prabhu PR, Pawlita M, et al, for the Indian HPV vaccine study group. Immunogenicity and HPV infection after one, two, and three doses of quadrivalent HPV vaccine in girls in India: a multicentre prospective cohort study. Lancet Oncol 2015; published online Dec 1. http://dx.doi.org/10.1016/S1470-2045(15)00414-3.
Appendix

Supplementary table 1. Mean MFI values of HPV 16, 18, 6 and 11 L1 antibodies on day 1 and at months 7, 18, 36 and 48 after the first dose among girls who received vaccination per protocol, and at months 12, 18 and 36 after the first dose among girls who did not have their eir complete vaccine schedules by default

| Vaccine doses received | No. samples | Geometric mean MFI (95% CI) | MFI ratio (95% CI)* (alternate dose/standard 3-dose at days 1, 60, 180) | No. (%) with MFI ≥ sero-conversion levels** | No. (%) with MFI ≥ than the lowest in standard 3-dose at that time point$ |
|-----------------------|-------------|-----------------------------|---------------------------------------------------------------------|--------------------------------------------|--------------------------------------------------|
| **Day 1**             |             |                             |                                                                     |                                            |                                                  |
| HPV 16 L1             |             |                             |                                                                     |                                            |                                                  |
| 3-dose group          | 1000        | 11 (10 - 12)                | 1·00                                                                |                                            | 46 (4·6)                                         |
| 2-dose group          | 937         | 9 (8 - 10)                  | 0·86 (0·74 - 0·99)                                                  |                                            | 52 (5·5)                                         |
| HPV 18 L1             |             |                             |                                                                     |                                            |                                                  |
| 3-dose group          | 1000        | 6 (5 - 7)                   | 1·00                                                                |                                            | 41 (4·1)                                         |
| 2-dose group          | 937         | 5 (4 - 5)                   | 0·81 (0·72 - 0·92)                                                  |                                            | 63 (6·7)                                         |
| HPV 6 L1              |             |                             |                                                                     |                                            |                                                  |
| 3-dose group          | 1000        | 24 (22 - 26)                | 1·00                                                                |                                            | 51 (5·1)                                         |
| 2-dose group          | 937         | 26 (24 - 29)                | 1·10 (0·97 - 1·25)                                                  |                                            | 44 (4·7)                                         |
| HPV 11 L1             |             |                             |                                                                     |                                            |                                                  |
| 3-dose group          | 1000        | 6 (6 - 7)                   | 1·00                                                                |                                            | 56 (5·6)                                         |
| 2-dose group          | 937         | 7 (6 - 7)                   | 1·03 (0·91 - 1·15)                                                  |                                            | 43 (4·6)                                         |
| **Month 7**           |             |                             |                                                                     |                                            |                                                  |
| HPV 16 L1             |             |                             |                                                                     |                                            |                                                  |


|                     | 3-dose (days 1, 60, 180) | 2-dose (days 1, 180) | HPV 18 L1 |                     | 3-dose (days 1, 60, 180) | 2-dose (days 1, 180) | HPV 6 L1 |                     | 3-dose (days 1, 60, 180) | 2-dose (days 1, 180) | HPV 11 L1 |                     | 3-dose (days 1, 60, 180) | 2-dose (days 1, 180) |
|---------------------|-------------------------|----------------------|-----------|---------------------|-------------------------|----------------------|----------|---------------------|-------------------------|----------------------|-----------|---------------------|-------------------------|----------------------|
|                     | 308 5460 (5195 - 5738)  | 317 6125 (5785 - 6485) | 308 (100-0) | 308 5460 (5195 - 5738) | 317 3068 (2812 - 3347) | 317 (100-0) | 308 5460 (5195 - 5738) | 317 3068 (2812 - 3347) | 317 (100-0) |
| 3-dose (days 1, 60, 180) | 308 5460 (5195 - 5738) | 317 6125 (5785 - 6485) | 308 (100-0) | 308 5460 (5195 - 5738) | 317 3068 (2812 - 3347) | 317 (100-0) | 308 5460 (5195 - 5738) | 317 3068 (2812 - 3347) | 317 (100-0) |
| 2-dose (days 1, 180) | 317 3068 (2812 - 3347) | 317 3068 (2812 - 3347) | 317 (100-0) | 317 3068 (2812 - 3347) | 317 3068 (2812 - 3347) | 317 (100-0) | 317 3068 (2812 - 3347) | 317 3068 (2812 - 3347) | 317 (100-0) |
|                     |                         |                      |           |                     |                         |                      |           |                     |                         |                      |           |                     |                         |                      |
| Month 12            |                         |                      |           |                     |                         |                      |           |                     |                         |                      |           |                     |                         |                      |
| HPV 16 L1           |                         |                      |           |                     |                         |                      |           |                     |                         |                      |           |                     |                         |                      |
| 2 doses (days 1, 60) | 471 437 (398 - 480)     | 528 106 (96 - 116)   | 430 (91-3) | 2 doses (days 1, 60) | 471 437 (398 - 480)     | 528 106 (96 - 116)   | 260 (49-2) | 2 doses (days 1, 60) | 471 437 (398 - 480)     | 528 106 (96 - 116)   | 260 (49-2) |
| A single dose       | 528 106 (96 - 116)      | 528 106 (96 - 116)   | 260 (49-2) | A single dose       | 528 106 (96 - 116)      | 528 106 (96 - 116)   | 260 (49-2) | A single dose       | 528 106 (96 - 116)      | 528 106 (96 - 116)   | 260 (49-2) |
| HPV 18 L1           |                         |                      |           |                     |                         |                      |           |                     |                         |                      |           |                     |                         |                      |
| 2 doses (days 1, 60) | 471 233 (214 - 254)     | 528 50 (45 - 55)     | 449 (95-3) | 2 doses (days 1, 60) | 471 233 (214 - 254)     | 528 50 (45 - 55)     | 304 (57-6) | 2 doses (days 1, 60) | 471 233 (214 - 254)     | 528 50 (45 - 55)     | 304 (57-6) |
| A single dose       | 528 50 (45 - 55)        | 528 50 (45 - 55)     | 304 (57-6) | A single dose       | 528 50 (45 - 55)        | 528 50 (45 - 55)     | 304 (57-6) | A single dose       | 528 50 (45 - 55)        | 528 50 (45 - 55)     | 304 (57-6) |
| HPV 6 L1            |                         |                      |           |                     |                         |                      |           |                     |                         |                      |           |                     |                         |                      |
| 2 doses (days 1, 60) | 471 509 (469 - 552)     | 528 167 (153 - 183)  | 373 (79-2) | 2 doses (days 1, 60) | 471 509 (469 - 552)     | 528 167 (153 - 183)  | 192 (36-4) | 2 doses (days 1, 60) | 471 509 (469 - 552)     | 528 167 (153 - 183)  | 192 (36-4) |
| A single dose       | 528 167 (153 - 183)     | 528 167 (153 - 183)  | 192 (36-4) | A single dose       | 528 167 (153 - 183)     | 528 167 (153 - 183)  | 192 (36-4) | A single dose       | 528 167 (153 - 183)     | 528 167 (153 - 183)  | 192 (36-4) |
| HPV 11 L1           |                         |                      |           |                     |                         |                      |           |                     |                         |                      |           |                     |                         |                      |
| 2 doses (days 1, 60) | 471 610 (562 - 662)     | 528 163 (149 - 179)  | 469 (99-6) | 2 doses (days 1, 60) | 471 610 (562 - 662)     | 528 163 (149 - 179)  | 466 (88-3) | 2 doses (days 1, 60) | 471 610 (562 - 662)     | 528 163 (149 - 179)  | 466 (88-3) |
| A single dose       | 528 163 (149 - 179)     | 528 163 (149 - 179)  | 466 (88-3) | A single dose       | 528 163 (149 - 179)     | 528 163 (149 - 179)  | 466 (88-3) | A single dose       | 528 163 (149 - 179)     | 528 163 (149 - 179)  | 466 (88-3) |
### Month 18

| Vaccine | Dose 1 | Dose 2 | Dose 3 | Mean | Std Dev | Median | Percent 1st Quartile | Percent 3rd Quartile |
|---------|--------|--------|--------|------|---------|--------|----------------------|----------------------|
| **HPV 16 L1** | | | | | | | | |
| 3-dose (days 1, 60, 180) | 313 | 1209 | (1105 - 1323) | 1.00 | | 255 | (53.6) | 273 | (57.4) |
| 2-dose (days 1, 180) | 314 | 1222 | (1116 - 1338) | 1.01 | (0.87 - 1.18) | 411 | (91.5) | 417 | (92.9) |
| 2 doses (days 1, 60) | 449 | 401 | (366 - 440) | 0.33 | (0.29 - 0.38) | 313 | (100.0) | |
| A single dose | 476 | 113 | (102 - 126) | 0.09 | (0.08 - 0.11) | 313 | (100.0) | |
| **HPV 18 L1** | | | | | | | | |
| 3-dose (days 1, 60, 180) | 313 | 377 | (337 - 422) | 1.00 | | 307 | (98.1) | 313 | (100.0) |
| 2-dose (days 1, 180) | 314 | 269 | (241 - 299) | 0.71 | (0.60 - 0.84) | 305 | (97.1) | 313 | (99.7) |
| 2 doses (days 1, 60) | 449 | 192 | (176 - 209) | 0.51 | (0.43 - 0.59) | 426 | (94.9) | 443 | (98.7) |
| A single dose | 476 | 46 | (40 - 51) | 0.12 | (0.10 - 0.14) | 259 | (54.4) | 347 | (72.9) |
| **HPV 6 L1** | | | | | | | | |
| 3-dose (days 1, 60, 180) | 313 | 986 | (900 - 1080) | 1.00 | | 296 | (94.6) | 313 | (100.0) |
| 2-dose (days 1, 180) | 314 | 830 | (756 - 911) | 0.84 | (0.73 - 0.97) | 292 | (93.0) | 313 | (99.7) |
| 2 doses (days 1, 60) | 449 | 476 | (440 - 514) | 0.48 | (0.42 - 0.55) | 357 | (79.5) | 431 | (96.0) |
| A single dose | 476 | 169 | (154 - 186) | 0.17 | (0.15 - 0.20) | 182 | (38.2) | 332 | (69.7) |
| **HPV 11 L1** | | | | | | | | |
| 3-dose (days 1, 60, 180) | 313 | 1327 | (1216 - 1449) | 1.00 | | 313 | (100.0) | 313 | (100.0) |
| 2-dose (days 1, 180) | 314 | 1328 | (1223 - 1443) | 1.00 | (0.87 - 1.15) | 314 | (100.0) | 314 | (100.0) |
| 2 doses (days 1, 60) | 449 | 530 | (489 - 574) | 0.40 | (0.35 - 0.45) | 449 | (100.0) | 449 | (100.0) |
| A single dose | 476 | 164 | (148 - 180) | 0.12 | (0.11 - 0.14) | 427 | (89.7) | 388 | (81.5) |

### Month 36

| Vaccine | Dose 1 | Dose 2 | Dose 3 | Mean | Std Dev | Median | Percent 1st Quartile | Percent 3rd Quartile |
|---------|--------|--------|--------|------|---------|--------|----------------------|----------------------|
| **HPV 16 L1** | | | | | | | | |
| 3-dose (days 1, 60, 180) | 271 | 221 | (197 - 247) | 1.00 | | 225 | (83.0) | 271 | (100.0) |
| HPV L1       | Month 48 | 2-dose (days 1, 180) | 2 doses (days 1, 60) | A single dose |
|-------------|----------|---------------------|---------------------|--------------|
| HPV 16 L1   |          | 278 163 (147 - 181) | 0.74 (0.63 - 0.87)  | 197 (70.9)   |
|             |          | 513 136 (126 - 147) | 0.62 (0.54 - 0.71)  | 324 (63.2)   |
|             |          | 510 72 (66 - 78)    | 0.33 (0.28 - 0.37)  | 166 (32.5)   |
| HPV 18 L1   |          | 278 117 (104 - 132) | 0.64 (0.53 - 0.76)  | 238 (85.6)   |
|             |          | 513 101 (93 - 109)  | 0.55 (0.47 - 0.64)  | 423 (82.5)   |
|             |          | 510 45 (41 - 49)    | 0.25 (0.21 - 0.29)  | 271 (53.1)   |
| HPV 6 L1    |          | 271 184 (162 - 208) | 1.00                | 249 (91.9)   |
|             |          | 278 117 (104 - 132) | 0.64 (0.53 - 0.76)  | 238 (85.6)   |
|             |          | 513 101 (93 - 109)  | 0.55 (0.47 - 0.64)  | 423 (82.5)   |
|             |          | 510 45 (41 - 49)    | 0.25 (0.21 - 0.29)  | 271 (53.1)   |
| HPV 11 L1   |          | 271 184 (162 - 208) | 1.00                | 249 (91.9)   |
|             |          | 278 117 (104 - 132) | 0.64 (0.53 - 0.76)  | 238 (85.6)   |
|             |          | 513 101 (93 - 109)  | 0.55 (0.47 - 0.64)  | 423 (82.5)   |
|             |          | 510 45 (41 - 49)    | 0.25 (0.21 - 0.29)  | 271 (53.1)   |
| Month 48    |          | 278 163 (147 - 181) | 0.74 (0.63 - 0.87)  | 197 (70.9)   |
|             |          | 513 136 (126 - 147) | 0.62 (0.54 - 0.71)  | 324 (63.2)   |
|             |          | 510 72 (66 - 78)    | 0.33 (0.28 - 0.37)  | 166 (32.5)   |
|              |          |                    |                     |              |
|              |          | 513 101 (93 - 109)  | 0.55 (0.47 - 0.64)  | 423 (82.5)   |
|              |          | 510 45 (41 - 49)    | 0.25 (0.21 - 0.29)  | 271 (53.1)   |
|              |          |                    |                     |              |
|              |          | 89 218 (181 - 262)  | 1.00                | 69 (77.5)    |
|              |          | 127 183 (160 - 209) | 0.84 (0.67 - 1.04)  | 101 (79.5)   |
| HPV 18 L1   |          | 89 206 (165 - 257)  | 1.00                | 82 (92.1)    |
|              |          |                    |                     |              |
| Dose Schedule          | MFI (95% CI) | MFI Ratio (95% CI) | MFI Ratio (95% CI) | MFI Ratio (95% CI) | MFI Ratio (95% CI) |
|------------------------|-------------|--------------------|--------------------|--------------------|--------------------|
| 2-dose (days 1, 180)   | 127         | 129                | (111 - 151)        | 0.63               | (0.49 - 0.82)      | 118 (92.9)        | 124 (97.6)        |

**HPV 6 L1**

| Dose Schedule          | MFI (95% CI) | MFI Ratio (95% CI) | MFI Ratio (95% CI) | MFI Ratio (95% CI) | MFI Ratio (95% CI) |
|------------------------|-------------|--------------------|--------------------|--------------------|--------------------|
| 3-dose (days 1, 60, 180) | 89         | 659                | (551 - 789)        | 1.00               | 77 (86.5)          | 89 (100.0)        |
| 2-dose (days 1, 180)   | 127         | 512                | (439 - 596)        | 0.78               | (0.61 - 0.98)      | 100 (78.7)        | 127 (100.0)       |

**HPV 11 L1**

| Dose Schedule          | MFI (95% CI) | MFI Ratio (95% CI) | MFI Ratio (95% CI) | MFI Ratio (95% CI) | MFI Ratio (95% CI) |
|------------------------|-------------|--------------------|--------------------|--------------------|--------------------|
| 3-dose (days 1, 60, 180) | 89         | 762                | (637 - 911)        | 1.00               | 89 (100.0)        | 89 (100.0)        |
| 2-dose (days 1, 180)   | 127         | 690                | (594 - 801)        | 0.91               | (0.72 - 1.14)      | 127 (100.0)        | 125 (98.4)        |

MFI: median fluorescence intensities; CI: confidence interval; HPV: human papilloma virus; * Other dose schedules were non-inferior to the 3-dose schedule for month 7, 18, 36 and 48 or to the incomplete 2 doses (Day 1, 60) for month 12 if the lower bound of the 95% CI for the MFI ratio was above 0.5 (2 times difference); ** The MFI sero-conversion levels for HPV 16, 18, 6 and 11 L1 antibodies were 240, 48, 100 and 41 respectively; $ The respective lowest MFI levels for HPV 16, 18, 6 and 11 L1 antibodies among the girls who received the standard 3 doses (Day 1, 60, 180) were 1011, 271, 521 and 1244 at 7 months, 90, 25, 100 and 61 at 18 months, 12, 11, 24 and 30 at 36, and 43, 20, 58 and 92 at 48 months after first dose.
Supplementary table 2. Geometric mean avidity index of MFI for HPV 16, 18, 6 and 11 L1 antibodies at 7 and 18 months after the first dose among girls who received vaccination per protocol, and those who did not have their complete vaccine schedules

| HPV type and dose             | No. Geometric mean | Geometric mean | Geometric mean avidity index* | Avidity index ratio (95% CI) | Alternate dose/3-dose³ |
|-------------------------------|--------------------|----------------|---------------------------|-----------------------------|------------------------|
| Received                      | samples            | 0M MFI         | 5M MFI                    | (% 95% CI)                  |                        |
| HPV 16 L1                     |                    |                |                           |                             |                        |
| 3-dose (days 1, 60, 180)      | 97                 | 1063           | 749                       | 70 (68 - 73)                | 1·00                   |
| 2-dose (days 1, 180)          | 99                 | 1758           | 1147                      | 65 (62 - 68)                | 0·93 (0·83 - 1·04)     |
| HPV 18 L1                     |                    |                |                           |                             |                        |
| 3-dose (days 1, 60, 180)      | 97                 | 660            | 540                       | 82 (79 - 85)                | 1·00                   |
| 2-dose (days 1, 180)          | 99                 | 907            | 724                       | 80 (75 - 85)                | 0·97 (0·87 - 1·09)     |
| HPV 6 L1                      |                    |                |                           |                             |                        |
| 3-dose (days 1, 60, 180)      | 97                 | 1590           | 1230                      | 77 (74 - 81)                | 1·00                   |
| 2-dose (days 1, 180)          | 99                 | 2216           | 1629                      | 74 (70 - 78)                | 0·95 (0·84 - 1·07)     |
| HPV 11 L1                     |                    |                |                           |                             |                        |
| 3-dose (days 1, 60, 180)      | 97                 | 1975           | 1694                      | 86 (82 - 90)                | 1·00                   |
| 2-dose (days 1, 180)          | 99                 | 2867           | 2306                      | 80 (76 - 85)                | 0·94 (0·83 - 1·06)     |
| Month 7                       |                    |                |                           |                             |                        |
| HPV 16 L1                     |                    |                |                           |                             |                        |
| 2 doses (days 1, 60)          | 142                | 158            | 109                       | 69 (66 - 73)                | 1·03 (0·95 - 1·12)     |
| A single dose                 | 130                | 46             | 34                        | 74 (68 - 80)                | 1·10 (1·01 - 1·19)     |

HPV 16 L1

| Month 18                      |                          |                |                           |                             |                        |
| 3-dose (days 1, 60, 180)      | 136                | 287            | 193                       | 67 (64 - 71)                | 1·00                   |
| 2-dose (days 1, 180)          | 139                | 335            | 221                       | 66 (63 - 70)                | 0·98 (0·90 - 1·07)     |
| 2 doses (days 1, 60)          | 142                | 158            | 109                       | 69 (66 - 73)                | 1·03 (0·95 - 1·12)     |
| A single dose                 | 130                | 46             | 34                        | 74 (68 - 80)                | 1·10 (1·01 - 1·19)     |
|          | Median (IQR) | Avidity Index Ratio | CI             |
|----------|--------------|---------------------|----------------|
| **HPV 18 L1** |              |                     |                |
| 3-dose (days 1, 60, 180) | 136 (114) | 87 76 (73 - 81) | 1·00           |
| 2-dose (days 1, 180) | 139 (101) | 76 75 (71 - 80) | 0·98 (0·90 - 1·08) |
| 2 doses (days 1, 60) | 142 (68)  | 52 76 (73 - 81) | 1·00 (0·91 - 1·10) |
| A single dose  | 130 (20)   | 17 85 (77 - 93) | 1·11 (1·01 - 1·22) |
| **HPV 6 L1**    |              |                     |                |
| 3-dose (days 1, 60, 180) | 136 (294) | 205 70 (66 - 74) | 1·00           |
| 2-dose (days 1, 180) | 139 (291) | 199 68 (64 - 73) | 0·98 (0·89 - 1·09) |
| 2 doses (days 1, 60) | 142 (160) | 112 70 (66 - 75) | 1·01 (0·92 - 1·12) |
| A single dose  | 130 (74)   | 48 65 (59 - 72) | 0·94 (0·85 - 1·04) |
| **HPV 11 L1**   |              |                     |                |
| 3-dose (days 1, 60, 180) | 136 (391) | 331 85 (81 - 89) | 1·00           |
| 2-dose (days 1, 180) | 139 (402) | 339 84 (80 - 89) | 0·99 (0·91 - 1·09) |
| 2 doses (days 1, 60) | 142 (188) | 154 82 (78 - 86) | 0·97 (0·89 - 1·06) |
| A single dose  | 130 (71)   | 63 88 (80 - 97) | 1·04 (0·95 - 1·14) |

MFI: median flow intensities; CI: confidence interval; HPV: human papilloma virus; * Proportion obtained from dividing treated MFI (at 5M) by untreated MFI (at 0M) and then multiplied by 100; CI: confidence interval;

Other dose schedules were non-inferior to the 3-dose standard schedule (Days 1, 60 and 180) if the lower bound of the 95% CI for the avidity index ratio was above 0·5 (2 times difference)
Supplementary table 3. Geometric mean neutralization titres of HPV 16, 18 and 6 L1 antibodies at 18 months after first dose among girls who received vaccination per protocol, and those who did not have their complete vaccine schedules

| HPV type | Samples tested | Samples with neutralization titres (%) | Geometric mean neutralization titres (95% CI) | Geometric mean ratio (95% CI)* | Alternate dose/3-dose |
|----------|----------------|----------------------------------------|---------------------------------------------|--------------------------------|-----------------------|
| HPV 16 L1 |                |                                        |                                             |                               |                       |
| 3-dose (Day 1, 60, 180) | 60 | 60 (100·0) | 9906 (7552 - 12995) | 1·00 |                       |
| 2-dose (Day 1, 180) | 59 | 59 (100·0) | 9893 (7754 - 12621) | 1·00 | (0·69 - 1·45) |
| 2 doses (Day 1, 60) | 60 | 60 (100·0) | 2311 (1773 - 3011) | 0·23 | (0·16 - 0·34) |
| A single dose | 58 | 56 (96·6) | 558 (416 - 750) | 0·06 | (0·04 - 0·08) |
| HPV 18 L1 |                |                                        |                                             |                               |                       |
| 3-dose (Day 1, 60, 180) | 60 | 59 (98·3) | 1951 (1403 - 2713) | 1·00 |                       |
| 2-dose (Day 1, 180) | 59 | 59 (100·0) | 819 (573 - 1170) | 0·42 | (0·27 - 0·65) |
| 2 doses (Day 1, 60) | 60 | 57 (95·0) | 431 (321 - 578) | 0·22 | (0·14 - 0·34) |
| A single dose | 58 | 39 (67·2) | 156 (113 - 216) | 0·08 | (0·05 - 0·13) |
| HPV 6 L1 |                |                                        |                                             |                               |                       |
| 3-dose (Day 1, 60, 180) | 60 | 60 (100·0) | 13936 (9623 - 20181) | 1·00 |                       |
| 2-dose (Day 1, 180) | 59 | 59 (100·0) | 10867 (7908 - 14934) | 0·78 | (0·50 - 1·21) |
| 2 doses (Day 1, 60) | 60 | 60 (100·0) | 3126 (2365 - 4131) | 0·22 | (0·15 - 0·35) |
| A single dose | 58 | 57 (98·3) | 787 (592 - 1046) | 0·06 | (0·04 - 0·09) |

CI: confidence interval; HPV: human papilloma virus; * Other dose schedules were non-inferior to the 3-dose schedule if the lower bound of the 95% CI for the MFI ratio was above 0·5 (2 times difference)
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| Site       | Name of the PI                                      | Number of enrolled girls | Number of recruited girls |
|------------|----------------------------------------------------|--------------------------|---------------------------|
| Barshi     | Dr. Sylla G. Malvi                                 | 8335                     | 7092                      |
| Ambilikkai | Dr. Pulikottil O. Esmy                             | 3499                     | 3300                      |
| Pune       | Dr. Smita Joshi                                    | 3720                     | 3018                      |
| Ahmedabad  | Dr Parimal Jivarajani (until Aug. 8, 2014)         | 1022                     | 1011                      |
|            | Dr Geeta Joshi (from Aug. 9, 2014)                |                          |                           |
| Delhi      | Dr Neerja Bhatla                                   | 1609                     | 1000                      |
| Hyderabad  | Dr Usha Rani Reddy Poli                            | 1168                     | 794                       |
| Mumbai     | Dr Surendra S. Shastri                             | 800                      | 514                       |
| Mizoram    | Dr Eric Zomawia                                    | 545                      | 500                       |
| Sikkim     | Dr Yogesh Verma                                    | 560                      | 500                       |
**Detailed procedures**

At each study site, dedicated health workers and nurses were recruited and trained to enumerate and interview the eligible girls for socio-demographic and reproductive information, explain the study to the participating girls and their parents, obtain informed consent, administer the vaccines, document adverse events, and carry out follow-up procedures including collection of blood and cervical cell samples. The study was explained to a number of medical practitioners in the study clusters to make them aware of how to manage and/or refer the vaccinated girls if any reported to them with suspected adverse events. A 24-hour committed telephone line was provided to the participants to contact a study clinician in case any need for urgent medical attention arose.

**Sample size calculation:**
The sample size for the study was calculated to allow observation of 16 cases per group of high-grade cervical intraepithelial neoplasia (CIN 2-3) related to HPV 16/18 infection by 60 months. The required sample size was determined under the following assumptions:

1. The average cluster size of 100 girls
2. Annual drop-out rate of 5%
3. For the intention-to-treat analysis, cumulative incidence rate of 1.3/10,000 in the three-dose group of CIN 2-3 lesions caused by HPV 16 and 18
4. The two doses of vaccine would be considered non-inferior to three doses if the increase in incidence of HPV 16/18-related CIN 2/3 or worse is less than three times the incidence within the three-dose group (i.e.&lt;5.4/10,000). Under assumptions about the incidence in an unvaccinated population\textsuperscript{13} with incidence of 40.7/10,000, this corresponds to a drop in absolute vaccine efficacy of less than 10 percentage points
5. A power of 80% and a one-sided 95% confidence (α=0.05)
6. Supposing that the cluster rates are log-normally distributed and that 95% of the clusters will have a true rate within a factor of 2 of the mean rate, using the incidence rate in the
unvaccinated population, the estimated intracluster correlation coefficient would be 0.0008.

7. Clusters of varying sizes with a coefficient of variation of 0.05

Under these assumptions, approximately 120 clusters and 12,000 girls (60 clusters and 6,000 girls per vaccination group) would be required.

However, a subgroup analysis in which the effect of the two- versus the three-dose will be compared in the age at entry categories 10-12, 12-15 and 16-18, is planned. Using Bonferroni adjustment,\(^{20-21}\) i.e. taking $\alpha=1.67\%$ (5%/3) as the level of significance for each of the subgroup analysis in order to be able to remain with a 5% level of significance for the overall effect, approximately 164 clusters and 16,400 girls (82 clusters and 8,200 girls per vaccination group) would be required for the subgroup analysis. This was calculated to allow observation of 22 CIN 2-3 cases per group related to HPV types 16 and 18 by 60 months.

Recruitment of the girls into the 2-dose and 3-dose groups and vaccination of eligible girls was initiated on 9 July 2009 and progressed satisfactorily until 8 April 2010, with more than 95% participation of the invited girls for vaccination, when the Indian authorities suspended all further recruitment and vaccination of subjects in all HPV vaccination trials in India, due to events unrelated to our study.

The suspension occasioned our having four groups of vaccinated girls: on days 1, 60 and 180 or more (three-dose group); on days one and 180 or more (two-dose group); on days one and 60 by default (two-dose/D group); and one dose only by default (one-dose/D group) (Figure 1). We continue to follow-up these cohorts of girls with different doses to evaluate the outcomes in terms of immunogenicity, the frequency of HPV infection and cervical neoplasia.

Each participant is visited annually in her household and her health, well-being and vital status enquired into. Details regarding marriage, medically significant events, pregnancy, ante- and post-natal events, delivery and migration are collected and documented through a network of social workers, medical care providers, hospitals and relatives. Appointments are fixed for blood
and cervical cell collection at designated periods.

To assess the sero-conversion, immunogenicity, antibody levels and durability of the immune response, blood samples were collected at baseline, 7, 12, 18, 24, 36, 48 and 60 months from a sample of the study population (Figure 1).

A sub-set of EDTA plasma at baseline, 7, 12, 18, 24, 36 and 48 months from a cohort of vaccinated girls have been analyzed using luminex-based Multiplex serology\textsuperscript{8, 9} to assess HPV-L1 binding antibodies against the major capsid protein L1 of vaccine types HPV 16, 18, 6 and 11 at the Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram, India, where a dedicated laboratory was established with technology-transfer and external quality assurance from the German Cancer Research Center (DKFZ), Heidelberg, Germany. Scientists from RGCB were trained at the DKFZ, Heidelberg, and the multiplex serology HPV-L1 antibody assays were carried out at RGCB by trained personnel, blinded to the study groups, under the technical supervision of experts from DKFZ. Briefly, fluorescence-coded bead sets (3000 beads per set per well) carrying different recombinant, affinity-purified HPV-L1 antigens were mixed and incubated with plasma diluted to 1:1000 in 96-well plates. After incubation and washing, biotinylated goat anti-human immunoglobulin antibody and subsequently the fluorescent reporter conjugate streptavidin-R-phycoerythrin were added and the reporter fluorescence of the beads was determined with a Luminex analyzer (Bioplex 200) and expressed as the median fluorescence intensity (MFI) of at least 100 beads per set per well. Final antigen specific net MFI values were generated by subtraction of GST-tag and individual bead background values. MFI as measure of antibody reactivities quantified by HPV multiplex serology are directly comparable to optical densities measured by enzyme-linked immunosorbent assay (ELISA)\textsuperscript{8} and MFI values for HPV16 in natural and vaccine-induced HPV16-specific antibody responses are strongly correlated with end-point titration titer in neutralization assay\textsuperscript{9} Sero-positivity cut-offs were calculated for each HPV type, based on the MFI values of serum samples obtained from the participants at baseline. The cut-off values were defined after allowing for 5% sero-positivity
among the total base-line samples. The immunogenicity measure was the geometric mean of MFI. \(^8, 9\)

Antibody avidity, which reflects the degree of antibody affinity maturation, was measured in a modification of the HPV-L1 genotype specific binding antibody assay described above. After the first washing step the beads with the antigen-antibody complexes were incubated for 15 minutes with the chaotropic agent urea at 5 M concentration or washing buffer alone and then the standard washing procedure continued. Under this high urea concentration low-avidity antibodies can detach from the antigen and are subsequently washed away which results in lower MFI values as compared to the incubation with buffer alone.

Neutralizing antibodies specific for neutralizing-epitopes in HPV-L1 protein were measured using a highly sensitive, automated, high-throughput pseudovirion-based neutralization assay (PBNA) with excellent repeatability and run-to-run reproducibility. \(^9\) Bovine papillomavirus (BPV) pseudovirion assays were run as control to verify that the test serum is not toxic to the cells, which can mimic neutralization. The Lower Limit of Quantitation (LLOQ) for the HPV-PBNA is a reciprocal dilution of 40. A sample was classified as sero-negative if the PBNA titre was <50; seropositive if the PBNA titre was \(\geq 50\) and \(\geq 2\) times the BPV titre; or sero-status indeterminate if the PBNA titre was \(\geq 50\) and <2 times the BPV titre.

Pelvic examination to collect cervical cell samples is being carried out in married girls 18 months after marriage or 6 months after delivery, whichever is earlier, and annually thereafter for 3 consecutive years.

A dedicated HPV testing laboratory was established at the RGCB, with technology transfer and quality assurance from the Infections and Cancer Biology Group at IARC and the DKFZ. The HPV genotyping method involved HPV type-specific E7 PCR bead-based multiplex genotyping (TS-MPG). \(^10, 11\) The multiplex HPV type-specific E7 PCR utilizes HPV type-specific primers
targeting the E7 region for the detection of 19 high-risk (HR) / probable HR-HPV types (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68\textsuperscript{a,b}, 70, 73, 82), and two low-risk (LR)-HPV types (HPV 6 and 11), with detection limits ranging from 10 to 1000 copies of the viral genome. The method was validated at RGCB under the supervision of scientists from IARC and the testing of cervical cell samples are carried out in RGCB.

The study has so far been monitored and evaluated by outcomes such as sero-conversion, comparison of the immunogenicity over a 48-month period after the first dose, and the frequency of incident and persistent vaccine targeted and non-targeted HPV infections in the different dose groups as the study progresses. In due course, we will document the frequency of CIN 2 and 3 caused by vaccine-targeted and non-targeted HPV types and, in the long-term, invasive cervical cancer incidence in the different dose groups over several years of follow-up by linking with population-based cancer registries.