Post-treatment HPV antibody kinetics in cervical cancer patients - Supplemental Material

Till Piontek[1§], Christoph Harmel[1§], Michael Pawlita[1], Katrin Carow[2], Juliane Schröter[1], Ingo B. Runnebaum[2], Matthias Dürst[2], Frederik Graw[3]*, Tim Waterboer[1*#]

[1] Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 242, 69120 Heidelberg

[2] Department of Gynaecology, Jena University Hospital, Am Klinikum 1, Haus F2, 07747 Jena

[3] Center for Modelling and Simulation in the Biosciences, BioQuant-Center, Heidelberg University, Im Neuenheimer Feld 267, 69120 Heidelberg

[§]indicates co-first authorship

*indicates co-senior authorship

[#] corresponding author (t.waterboer@dkfz.de)

Number of Main Tables: 4

Number of Main Figures: 3

Number of Supplementary Tables: 7

Number of Supplementary Figures: 8

Supplementary tables and figures

Suppl. Table 1: HPV18 serology and tumour HPV DNA status at baseline

	DNA status[1]	
Seropositive for	**HPV16 pos. (n=68)**	**HPV18 pos. (n=26)**
HPV18 E6	3 (4.4)	15 (57.7)
HPV18 E7	6 (8.8)	13 (50.0)
HPV18 L1	17 (25.0)	7 (26.9)

[1]One patient excluded due to double HPV16 and 18 positivity.

Suppl. Table 2: HPV18 serology during follow-up (n=173)

Antigen	Seropositive n (%)	Decreasing n (%)[1]	Stable or increasing n (%)[1]
HPV18 E1	50 (28.9)	9 (18.0)	41 (82.0)
HPV18 E2	27 (15.6)	7 (25.9)	20 (74.1)
HPV18 E4	14 (8.1)	1 (7.1)	13 (92.9)
HPV18 E6	22 (12.7)	4 (18.2)	18 (81.8)
HPV18 E7	22 (12.7)	9 (41.0)	13 (59.0)
HPV18 L1	38 (22.0)	6 (15.8)	32 (84.2)

[1] A decreasing trend shows reduction in antibody reactivity (MFI) of at least 50% of the baseline MFI within the first 18 months. Every other trend is considered to be either stable or increasing. Of the 184 patients, 11 patients who underwent laparoscopic staging without surgery were excluded in this analysis.

Suppl. Table 3: Seroreversion of seropositive patients

	All (n=173)	Among seropositives
Antigen	**Seropositive n (%)**	**Seroreversion n (%)**
HPV16 E6	67 (38.7)	13 (19.4)
HPV16 E7	50 (28.9)	13 (26.0)
HPV16 L1	47 (27.2)	5 (10.6)
HPV18 E6	22 (12.7)	7 (31.8)
HPV18 E7	22 (12.7)	7 (31.8)
HPV18 L1	38 (22.0)	7 (18.4)

Suppl. Table 4: Serological response to HPV16 with regard to follow-up status (n=173)

		Among seropositives		
Antigen	**Seropositive n (%)**	**Decreasing n (%)[1]**	**Stable or increasing n (%)[1]**	**No short-term follow-up n (%)[2]**
Relapse during serological follow-up (n=41)				
HPV16 E1	14 (34.2)	5 (35.7)	5 (35.7)	4 (28.6)
HPV16 E2	9 (22.0)	3 (33.3)	5 (55.6)	1 (11.1)
HPV16 E4	14 (34.2)	6 (42.9)	5 (35.7)	3 (21.4)
HPV16 E6	16 (39.0)	6 (37.5)	6 (37.5)	4 (25.0)
HPV16 E7	11 (26.8)	8 (72.7)	1 (9.1)	2 (18.2)
HPV16 L1	15 (36.6)	6 (40.0)	7 (46.7)	2 (13.3)
Relapse not during serological follow-up (n=22)				
HPV16 E1	7 (31.8)	3 (42.9)	4 (57.1)	0 (0.0)
HPV16 E2	4 (18.2)	1 (25.0)	3 (75.0)	0 (0.0)
HPV16 E4	5 (22.7)	3 (60.0)	2 (40.0)	0 (0.0)
HPV16 E6	13 (59.1)	4 (30.8)	7 (53.8)	2 (15.4)
HPV16 E7	10 (45.5)	5 (50.0)	3 (30.0)	2 (20.0)
HPV16 L1	7 (31.8)	1 (14.3)	5 (71.4)	1 (14.3)
No relapse (n=110)				
HPV16 E1	24 (21.8)	6 (25.0)	17 (70.8)	1 (4.2)
HPV16 E2	13 (11.8)	6 (46.2)	6 (46.2)	1 (7.7)
HPV16 E4	29 (26.4)	7 (24.1)	19 (65.5)	3 (10.3)
HPV16 E6	40 (36.4)	18 (45.0)	20 (50.0)	2 (5.0)

HPV16 E7	30 (27.3)	20 (66.7)	8 (26.7)	2 (6.7)
HPV16 L1	23 (20.9)	5 (21.7)	14 (60.9)	4 (17.4)

[1] A decreasing trend shows reduction in antibody reactivity (MFI) of at least 50% of the baseline MFI within the first 18 months. Every other trend is considered to be either stable or increasing. Of the 184 patients, 11 patients who underwent laparoscopic staging without surgery were excluded in this analysis.

[2] Patients without short-term follow-up samples, i.e. those with the first follow-up sample after more than 18 months, were analyzed separately

Suppl. Table 5: Parameter estimates of the non-linear mixed effect models for the 2-phase decay model for the antigens HPV16 E6 and E7. For each antigen, the parameter estimates and relative standard errors (r.s.e.) are given, as well as the AIC value of the model. In addition, the corresponding mean estimates and standard errors of the time to 50% reduction of the individual antibody levels based on the predicted dynamics, *A(t)*, for each patient are presented. For the individual patient estimates see Suppl. Tables 6 and 7. Please note that parameter estimates for this model are generally quite unstable (r.s.e. >50%).

	Parameter		**HPV16 E6**		**HPV16 E7**	
		(unit)	**estimate**	**r.s.e. (in %)**	**estimate**	**r.s.e. (in %)**
2-phase decay	Decay rate	λ (d^{-1})	0.218	71.16	0.266	64.61
	Antibody production	α (d^{-1})	0.057	71.78	0.072	67.99
	Plasma cell decay	δ (d^{-1})	3×10^{-4}	42.37	1.3×10^{-3}	11.52
		AIC	-134.49		-237.90	
			mean	**s.e.**	**mean**	**s.e**
	Time to 50% reduction in individual antibody titers A(t)	$t_{1/2}$ (d)	53.22	32.94	86.24	22.33

Suppl. Table 6: Individual parameter estimates for HPV16 E6

	HPV16 E6		
	power-law		
id	$\tilde{\lambda}$	**c**	$t_{1/2}$
2	0,225	1,101	20,542
6	0,412	1,098	4,281
12	0,158	1,106	79,701
26	0,267	1,091	12,290
45	0,289	1,096	9,867
52	0,323	1,092	7,484
56	0,437	1,083	3,797
62	0,107	1,080	656,498
69	0,386	1,079	4,937
72	0,171	1,101	56,561
82	0,248	1,097	15,228
93	0,202	1,092	29,713
96	0,352	1,084	6,096
98	0,250	1,092	14,892
99	0,414	1,087	4,256
100	0,164	1,091	68,238
107	0,377	1,094	5,185
115	0,289	1,083	9,938
118	0,182	1,108	43,513
120	0,116	1,091	391,828
124	0,264	1,086	12,663
125	0,194	1,093	34,554
128	0,365	1,091	5,601
137	0,281	1,086	10,690

153	0,204	1,084	28,919
161	0,220	1,096	22,387
172	0,204	1,088	29,025
189	0,362	1,080	5,709

2-phase decay

id	λ	δ	α	$t_{1/2}$
2	0,2056	0,0003	0,0616	6,085
6	0,3332	0,0003	0,0460	2,604
12	0,1567	0,0003	0,0755	20,737
26	0,2345	0,0003	0,0573	4,621
45	0,2388	0,0003	0,0551	4,394
52	0,2881	0,0003	0,0502	3,228
56	0,3278	0,0003	0,0441	2,630
62	0,1278	0,0003	0,0784	690,707
69	0,2989	0,0003	0,0494	3,057
72	0,1804	0,0003	0,0655	8,502
82	0,2339	0,0003	0,0581	4,675
93	0,1936	0,0003	0,0660	7,336
96	0,3234	0,0003	0,0530	2,818
98	0,2292	0,0003	0,0586	4,856
99	0,3068	0,0003	0,0461	2,892
100	0,1622	0,0003	0,0698	12,847
107	0,3124	0,0003	0,0493	2,883
115	0,2678	0,0003	0,0547	3,697
118	0,1829	0,0003	0,0652	8,180
120	0,1229	0,0003	0,0745	649,099
124	0,2360	0,0003	0,0614	4,770
125	0,1914	0,0003	0,0647	7,339
128	0,3002	0,0003	0,0490	3,032

137	0,2107	0,0003	0,0603	5,718
153	0,2057	0,0003	0,0622	6,121
161	0,2053	0,0003	0,0603	5,993
172	0,1819	0,0003	0,0644	8,156
189	0,2958	0,0003	0,0519	3,152

Suppl. Table 7: Individual parameter estimates for HPV16 E7

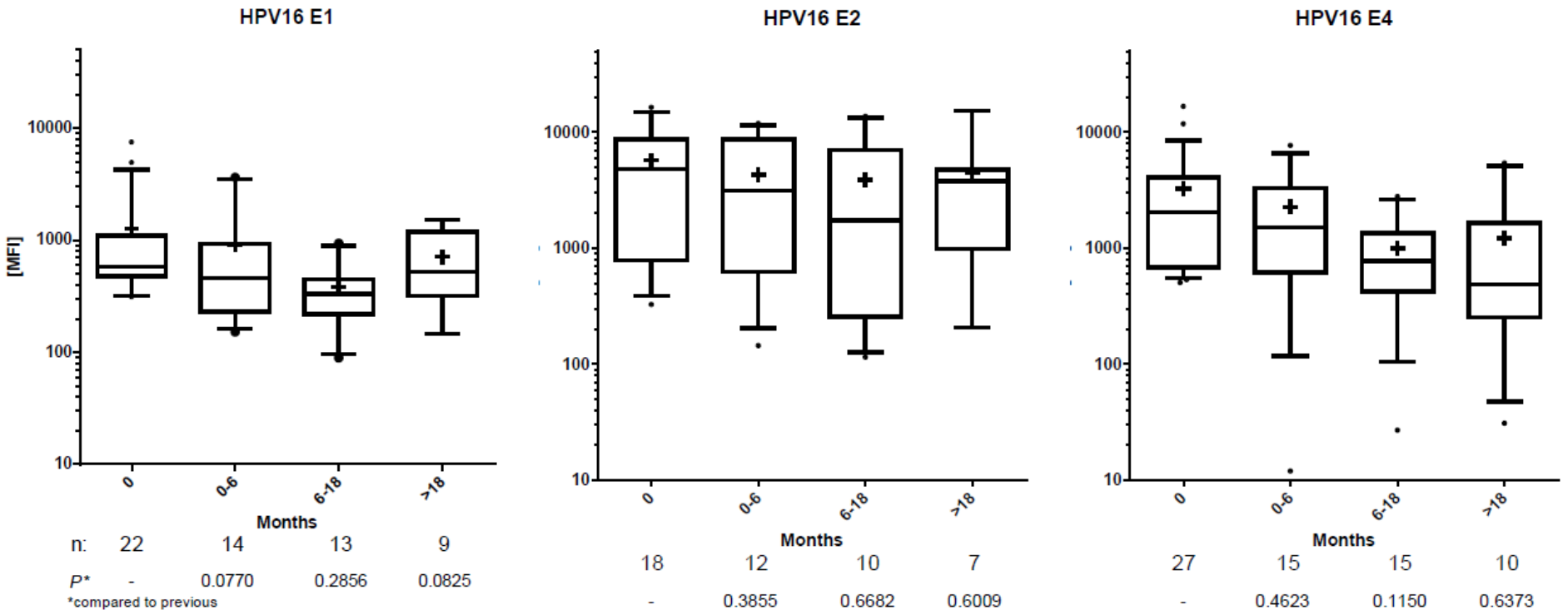
	HPV16 E7		
	power-law		
id	$\tilde{\lambda}$	**c**	$t_{1/2}$
2	0,249	1,403	14,822
6	0,618	1,397	1,671
8	0,136	1,385	161,865
12	0,169	1,418	58,319
13	0,264	1,392	12,365
22	0,291	1,402	9,468
31	0,553	1,393	2,106
44	0,415	1,366	3,939
56	0,433	1,404	3,545
63	0,208	1,414	26,631
72	0,168	1,405	60,816
74	0,175	1,399	51,172
79	0,519	1,427	2,376
80	0,438	1,409	3,467
81	0,456	1,368	3,210
82	0,533	1,403	2,264
86	0,110	1,400	543,184
96	0,363	1,389	5,359
99	0,587	1,379	1,878
101	0,871	1,370	0,846
102	0,205	1,397	27,796

111	0,121	1,395	300,729
120	0,182	1,394	43,572
123	0,274	1,390	11,135
124	0,341	1,385	6,268
129	0,130	1,427	201,585
137	0,227	1,389	19,732
140	0,177	1,402	48,279
153	0,167	1,389	62,324
161	0,151	1,394	98,331
171	0,676	1,372	1,416
172	0,192	1,391	35,534
180	0,198	1,391	31,572

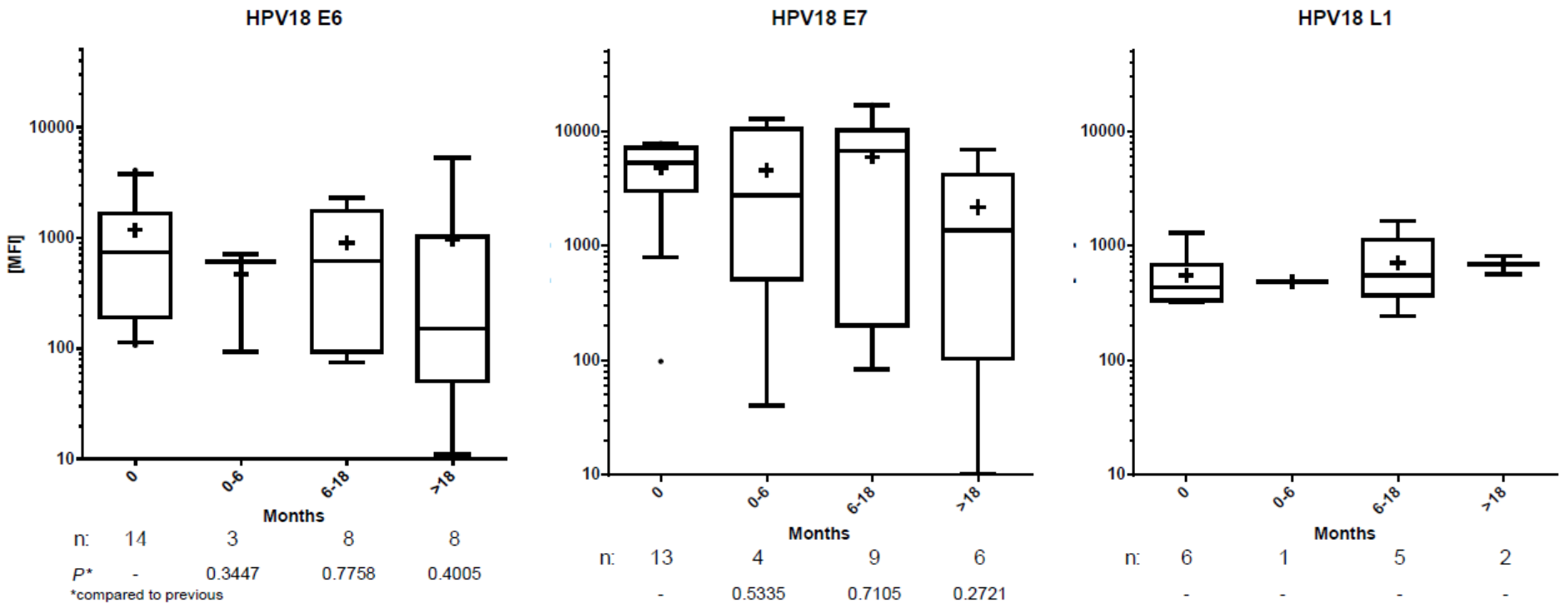
2-phase decay

id	λ	δ	α	$t_{1/2}$
2	0,2667	0,0013	0,0813	4,746
6	0,2759	0,0013	0,0294	2,972
8	0,2681	0,0013	0,1781	222,491
12	0,2791	0,0013	0,1442	29,039
13	0,2613	0,0013	0,0994	6,242
22	0,2709	0,0013	0,0809	4,591
31	0,2769	0,0013	0,0238	2,858
44	0,2817	0,0013	0,0464	3,237
56	0,2826	0,0013	0,0427	3,145
63	0,2690	0,0013	0,1078	6,617
72	0,2649	0,0013	0,1608	153,073
74	0,2854	0,0013	0,1839	198,596

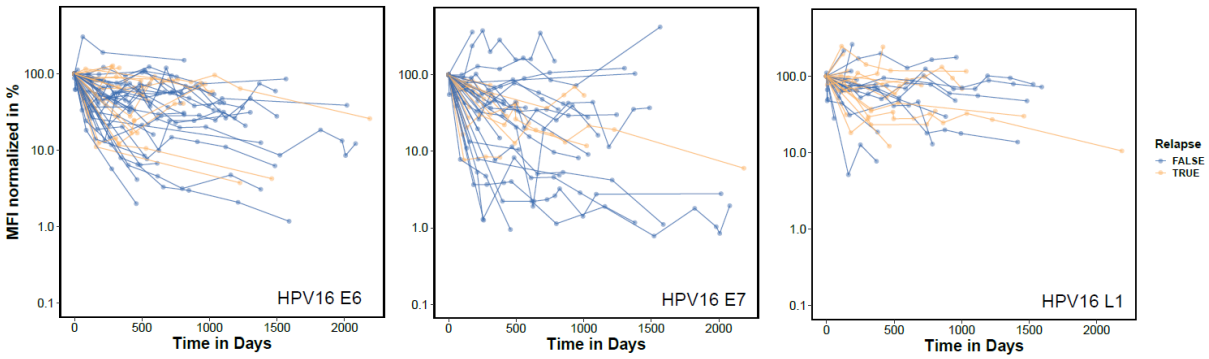
79	0,2616	0,0013	0,0260	3,096
80	0,2708	0,0013	0,0335	3,120
81	0,2741	0,0013	0,0357	3,119
82	0,2876	0,0013	0,0221	2,712
86	0,2732	0,0013	0,2381	430,596
96	0,2797	0,0013	0,0463	3,268
99	0,2847	0,0013	0,0299	2,871
101	0,2831	0,0013	0,0187	2,707
102	0,2530	0,0013	0,1614	191,361
111	0,2612	0,0013	0,1960	316,112
120	0,2600	0,0013	0,1549	138,191
123	0,2759	0,0013	0,0623	3,757
124	0,2794	0,0013	0,0487	3,327
129	0,2630	0,0013	0,2237	412,597
137	0,2676	0,0013	0,1044	6,345
140	0,2602	0,0013	0,1674	197,423
153	0,2640	0,0013	0,1701	198,731
161	0,2599	0,0013	0,1816	261,217
171	0,2841	0,0013	0,0316	2,910
172	0,2725	0,0013	0,1377	17,821
180	0,2600	0,0013	0,1051	6,959



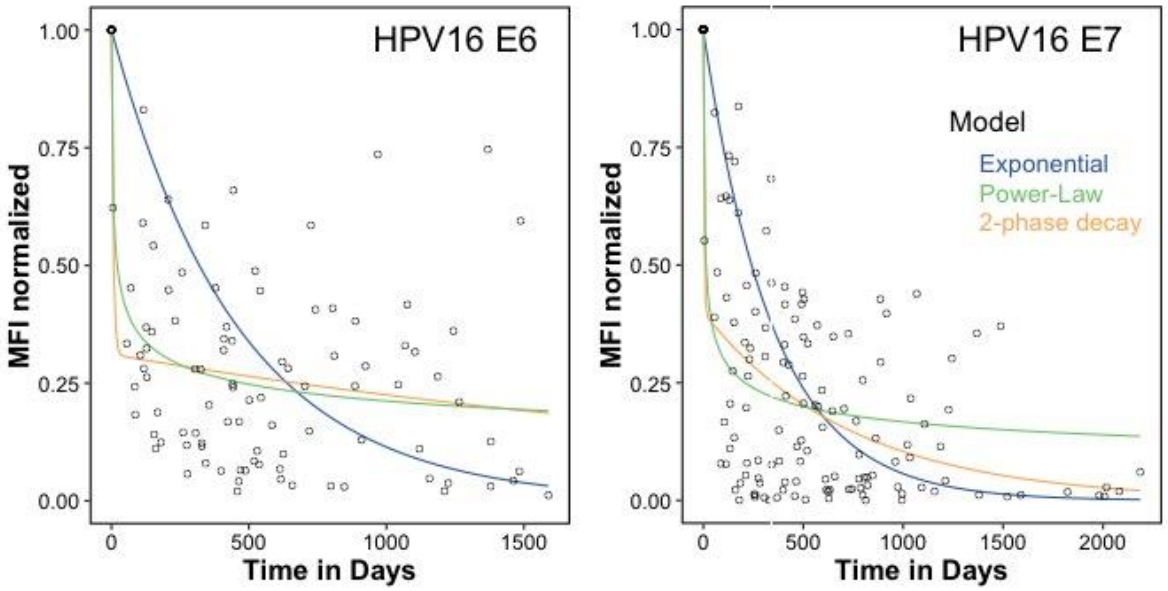
Suppl. Figure 1: Antibody decay of baseline HPV16 DNA-positive and seropositive cases. The three panels show antibody reactivity to HPV16 proteins E1, E2, E4 during follow-up. Serial serum samples were categorized as follows: baseline (month 0); the sample collected closest to 6 months follow-up (months 1-6); the sample collected closest to 18 months follow-up (months 7-18); the last follow-up sample available (>18 months). Whiskers represent 90th and 10th percentiles, respectively; medians are indicated by horizontal lines; means are displayed by crosses. Patient numbers with sera available at the given time points vary due to heterogeneous follow-up sampling. P values indicate statistical significance compared to previous category.



Suppl. Figure 2: Antibody decay of baseline HPV18 DNA-positive and seropositive cases. The three panels show antibody reactivity to HPV18 proteins E6, E7, L1 during follow-up. Serial serum samples were categorized as follows: baseline (month 0); the sample collected closest to 6 months follow-up (months 1-6); the sample collected closest to 18 months follow-up (months 7-18); the last follow-up sample available (>18 months). Whiskers represent 90th and 10th percentiles, respectively; medians are indicated by horizontal lines; means are displayed by crosses. Patient numbers with sera available at the given time points vary due to heterogeneous follow-up sampling. P values indicate statistical significance compared to previous category.



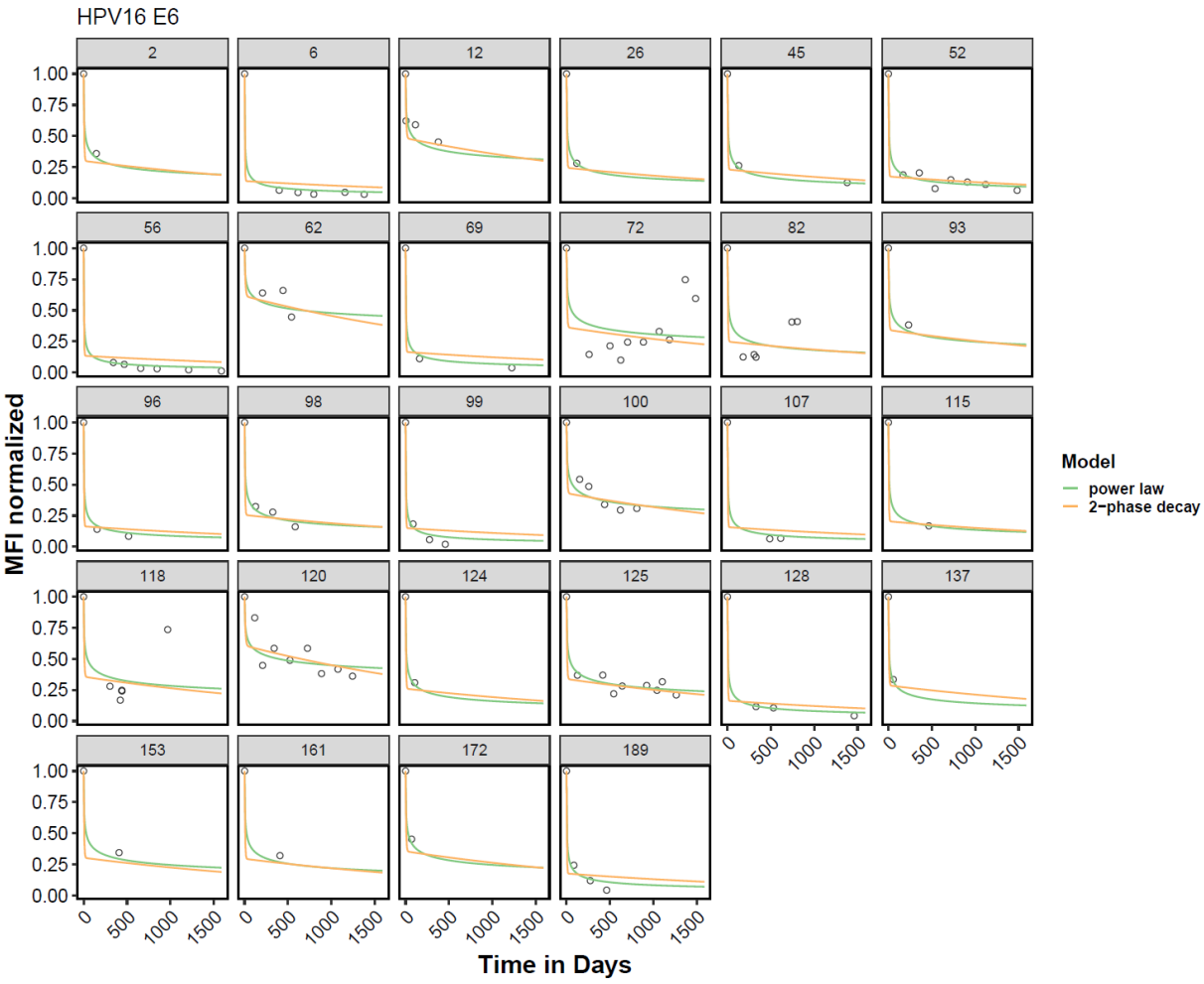
Suppl. Figure 3: Changes in HPV16 E6, E7 and L1 MFI levels for baseline seropositive cases over time by relapse status. Relapse cases are colored yellow and non-relapse cases blue. Please note that in this case all patients experiencing a relapse during or after follow-up were considered as relapse. Four patients for HPV16 E7 were excluded from the corresponding plot since they showed normalized MFI values of either >500% or <0.1%.



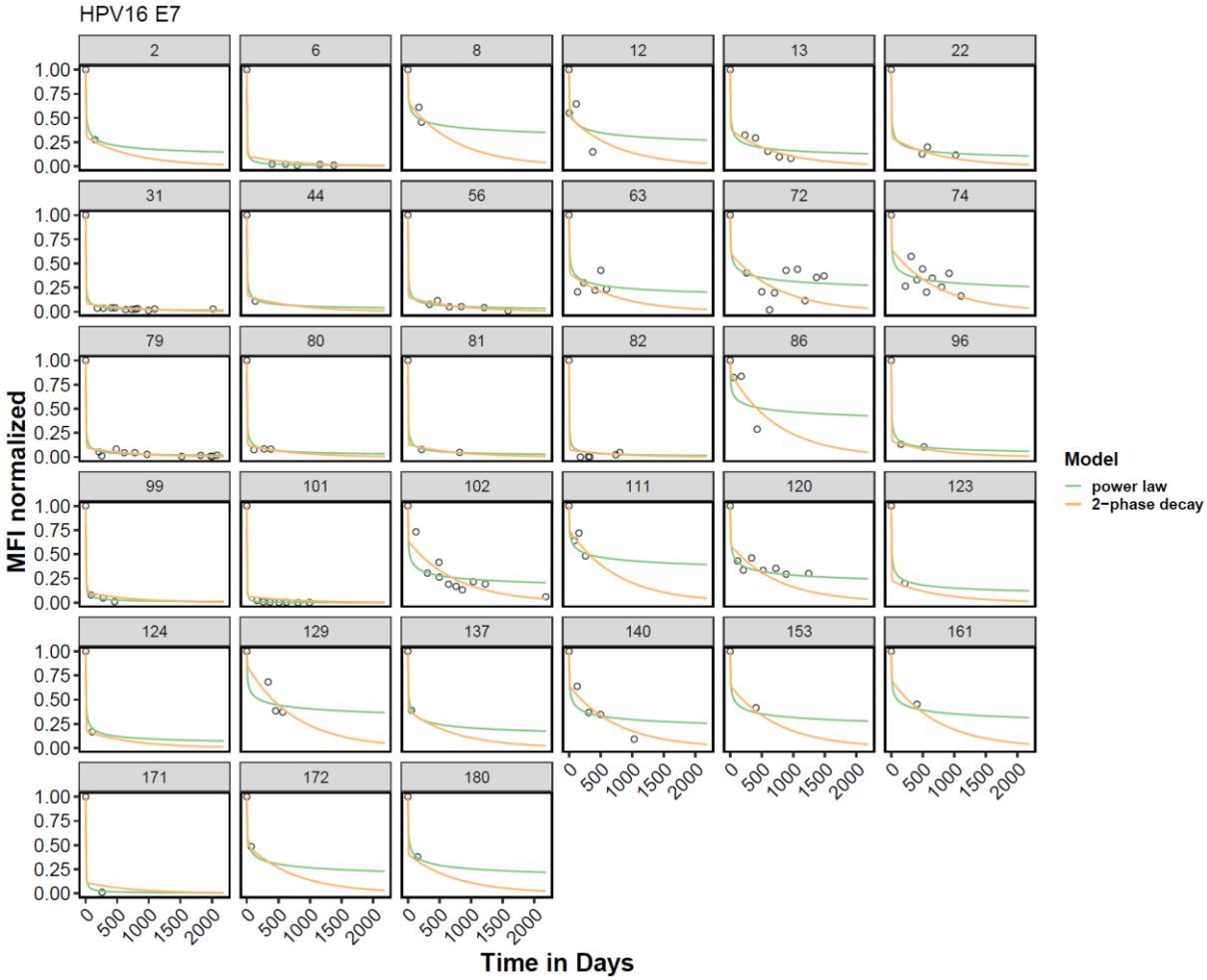
	Parameter		HPV16 E6		HPV16 E7	
		(unit)	estimate	s.e.	estimate	s.e.
exponential	Antibody decay	λ (d^{-1})	0.002	1×10^{-4}	0.003	2×10^{-4}
		AIC	351.76		643.20	
power-law	Antibody decay	$\tilde{\lambda}$ (d^{-1})	0.225	0.011	0.259	0.012
	Scaling constant	θ (d)	1.001	0.137	1.0	0.113
		AIC	-92.12		-115.86	
2-phase decay	Antibody decay	λ (d^{-1})	0.158	0.106	0.271	0.218
	Antibody production	α (d^{-1})	0.049	0.034	0.108	0.090
	Plasma cell decay	δ (d^{-1})	3×10^{-4}	2×10^{-4}	0.001	3×10^{-4}
		AIC	-87.90		-118.34	

Suppl. Figure 4: Individual measurements and model predictions for HPV16 E6 and E7. The analysis includes those patients that had been classified as having decreasing antibody kinetics, and neglects individual patient kinetics to identify appropriate model structures based on the population trend. Three different models including the exponential (blue), power-law (green) and 2-phase decay (orange) model are fitted to the data with parameter estimates and corresponding standard error (s.e.) shown in the corresponding table. Power-

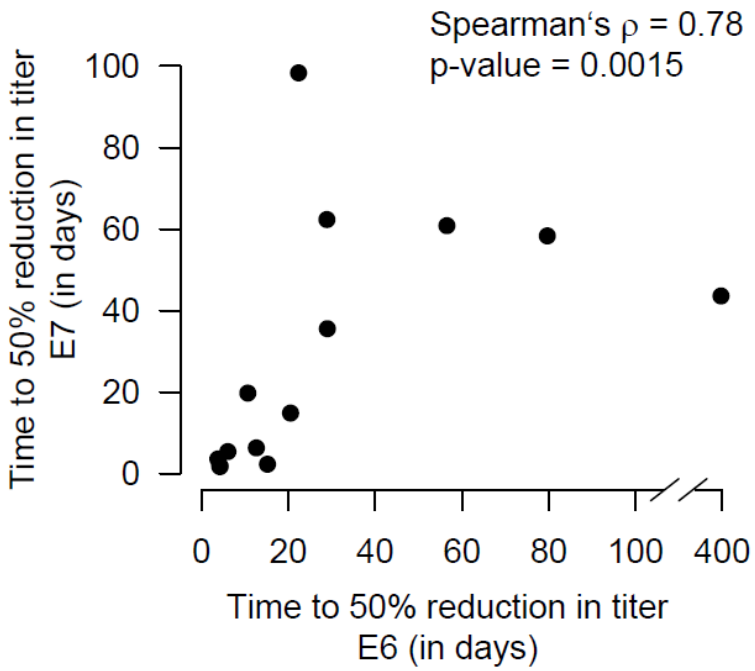
law and 2-phase decay model provide a substantially better fit to the data than the simple exponential model (compare AIC values).



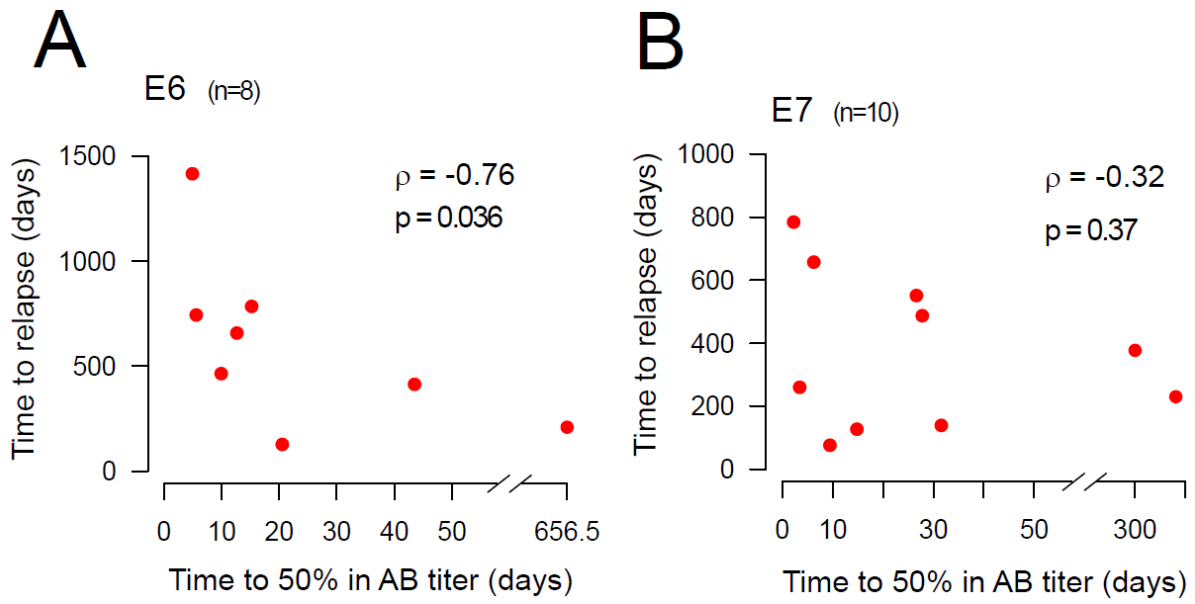
Suppl. Figure 5: Individual measured (circles) and predicted (lines) antibody kinetics for the 28 patients classified with decreasing dynamics for HPV16 E6. Model predictions for each patient based on the fits obtained by the non-linear mixed effects model are shown for the power-law (green) and 2-phase decay (orange) model. Individual parameter estimates are shown in Suppl. Table 6.



Suppl. Figure 6: Individual measured (circles) and predicted (lines) antibody kinetics for the 33 patients classified with decreasing dynamics for HPV16 E7. Model predictions for each patient based on the fits obtained by the non-linear mixed effects model are shown for the power-law (green) and 2-phase decay (orange) model. Individual parameter estimates are shown in Suppl. Table 7.



Suppl. Figure 7: Correlation of time to 50% reduction in antibody titers for HPV 16 E6 and E7. Correlation in the time to 50% reduction in antibody titers for HPV 16 E6 and E7 as predicted by the mathematical model for all patients that are classified as decreasing in both antigens, i.e., E6 and E7 (n=14).



Suppl. Figure 8: Time to relapse vs. time to 50% reduction in antibody titer.
Correlation between the time to reach 50% reduction in antibody titers and the time to relapse for E6 (A) and E7 (B) in patients showing decreasing antibody titers and experiencing a relapse. Please note that in this case all patients experiencing a relapse during or after follow-up were considered as relapse.